Original Article

P57^{kip2} Immunohistochemical Marker as a Diagnostic tool for Cases of Hydatidiform Moles in a Tertiary Health Facility in Southwestern Nigeria

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Abstract

Background: Hydatidiform mole (HM) is the most common gestational trophoblastic disease. P57^{kip2} has been reported to be helpful in differentiating between partial and complete HMs. **Objectives:** The study aims to evaluate the P57^{kip2} immunohistochemical (IHC) marker as a useful ancillary investigation to differentiate complete hydatidiform mole (CHM) from partial hydatidiform mole (PHM). **Materials and Methods:** A retrospective study of all histologically diagnosed HM cases over a 20 year period was undertaken. Clinicopathological parameters were extracted from the surgical day book and medical record archives. Archival haematoxylin- and eosin-stained slides and formalin-fixed paraffin-embedded tissue blocks of all cases of HM diagnosed within the study period were retrieved and reviewed. Cases of HM were reclassified using the P57^{kip2} IHC marker. The data obtained were analysed using the SPSS version 23. **Results:** One hundred cases of HMs were studied. CHM accounted for 68%, while PHM accounted for the remaining 32%. The incidence of HM was 2.98 cases per 1000 deliveries. The ratio of CHM to PHM was found to be 2.1:1. Seventy-two per cent of the cases were diagnosed in the first trimester, while the remaining 28% were diagnosed in the second trimester of pregnancy. Based on the P57^{kip2} IHC staining pattern, HM cases were finally reclassified into 68 cases of CHM and 32 cases of PHM. The age range for all the HM cases was 18–50 years with the majority of the cases seen in the third and fourth decades of life. **Conclusion:** P57^{kip2} could be useful as an ancillary investigation in confirming the diagnosis of CHM and differentiating it from PHM, particularly in difficult and challenging cases.

Keywords: Hydatidiform moles, Ibadan, Nigeria, P57kip2 immunohistochemical marker, partial hydatidiform mole

Introduction

Hydatidiform mole (HM) is part of a group of diseases classified as a gestational trophoblastic disease (GTD), which originates in the placenta and has potential to locally invade the uterus and metastasise.[1] HM is made up of two distinct entities, complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM). The invasive mole could be either a complete mole or partial mole that invades into the myometrium without intervening decidual tissue.[1]

The diagnosis of HM is mainly a clinicopathologic correlation including abnormal uterine bleeding, presence or absence of foetal parts, disproportionate gestational age, fundal height higher than normal and elevated beta-hCG levels. P57^{kip2} is the gene product that is paternally imprinted, maternally expressed. It is located on chromosome 11p15.5. Nuclear P57^{kip2} expression is detected in the villous cytotrophoblasts and stromal cells in normal placenta and partial HMs but completely absent in villous cytotrophoblasts and stromal cells of complete HMs. The negative nuclear P57^{kip2} expression in villous cytotrophoblasts and stromal cells in CHM, in contrast to other hydropic placentas, provides a valuable diagnostic tool that could be used in difficult cases.[1,2]

The incidence of HMs varies widely across different regions of Nigeria, Africa and the world.[3-10] This could be associated

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with some peculiar geographical factors, pregnancy-related risks, unknown environmental agents and genetic factors.[3] Patients with CHM usually tend to be above 30 years of age and are more likely to have diets deficient in Vitamin A precursors.[3] Many studies have reported intra- and inter-observer variability in the histologic diagnosis of HMs.[1,2] Hence, there is a need for ancillary investigations to be done to make the diagnosis more precise and accurate because of the prognostic implications. This study is aimed at evaluating the utility of the P57^{kip2} immunohistochemical (IHC) marker in the differential diagnosis of complete versus partial HMs in a black population and adds to the body of knowledge on this subject.

Materials and Methods

Ethical approval

Ethical approval for this study was obtained from the Joint University of Ibadan and University College Hospital, Ibadan Ethical Review Committee, with approval number: NHREC/05/01/2008a. The date of approval was 28th August 2018. The certificate of ethical clearance has been attached to the manuscript. The commencement date of data collection was 4th September 2018. The completion date of data collection was 20 November 2019. The period studied was between 1st January 1997 and 31st December 2016.

Study design

The study was a 20-year retrospective hospital-based study carried out in a tertiary hospital facility in Southwestern Nigeria. The total number of deliveries in the hospital over the study period was obtained from the labour ward register and Medical Records Department archives. Archival haematoxylin and eosin (H and E)-stained glass slides and corresponding formalin-fixed paraffin-embedded (FFPE) tissue blocks of all cases with a histological diagnosis of a HM within the study period were retrieved. These cases were reviewed by three pathologists and classified into specific histological subtypes. Cases in which there was difficulty using morphological criteria to differentiate between CHM and PHM were classified as equivocal hydatidiform moles (EHMs).

Inclusion criteria

Archival H and E-stained glass slides and corresponding FFPE tissue blocks of all cases with a histological diagnosis of a HM within the study period.

Exclusion criteria

Cases with missing histopathology glass slides and paraffin-embedded tissue blocks.

Sample size determination

The sample size of 100 was determined using the Leslie Fisher's formula for calculating minimal sample size, i.e., $n = z^2pq/d^2$.[3]

where:

n = minimum sample size

z = percentage point of standard normal distribution curve, which corresponds to 95% confidence interval. z = 1.96

p = prevalence rate of HM in Northern Nigeria is 5.7%[3]

q = complimentary probability; $q = 0.943$

d = degree of precision at 95% confidence limit; $d = 5\% = 0.05$.

By substituting these values into the formula (and using a prevalence of 5.7% of HM),

$n = (1.96)\ 2 \times 0.057 \times 0.943/0.0025 = 83$.

An attrition rate of 10% was assumed to account for anticipated dropout. This brought the total sample size to = calculated sample size (83) ÷ 1 − attrition rate = 83 ÷ 1− 0.1 = 83/0.9 = 92.

This was approximated to a minimum sample size of 100 used for this study.

Immunohistochemical staining

IHC staining for human P57^{kip2} protein was performed on representative sections from the tissue blocks of histologically diagnosed cases of HM including equivocal and unequivocal cases using the rabbit monoclonal antibody against the P57^{kip2} protein (Clone EP2515Y obtained from Abcam PLC., United Kingdom). The IHC staining was carried out using the heat-induced epitope retrieval method followed by a standard streptavidin–biotin peroxidase complex technique (MRH534 L obtained from Biocare Medical, USA). Positive reactivity was interpreted only when distinct nuclear staining was identified. As in a previous study, decidual stromal cells and syncytiotrophoblastic cells were used as reliable internal positive and negative controls, respectively, in each case.[11] Immunopositivity was recorded separately in the different cellular components, such as villous cytotrophoblast, villous intermediate trophoblast, villous syncytiotrophoblasts, villous stromal cells and maternal decidual cells.

Interpretation of results

The nuclear expression of P57^{kip2} in the villous stromal cells and cytotrophoblasts was interpreted as positive when at least 10% of the cells show nuclear positivity. A positive result was interpreted as consistent with a diagnosis of PHM. The staining was interpreted as negative when the villous stromal cells and cytotrophoblasts are either entirely negative or demonstrated only limited and weak nuclear expression (nuclear staining in <10% of the cytotrophoblasts and villous stromal cells). Cases showing cytoplasmic staining instead of the expected nuclear staining were also considered to be negative. A negative result was also interpreted as consistent with a diagnosis of CHM.

Data analysis

The data were analysed using the Statistical Package for Social Sciences (SPSS) Chicago, IL State, USA (SPSS) software version 23 (IBM Corporation, SPSS Statistics Inc., USA, 2014). The results were subsequently presented in tables, bar charts, pie charts, figures, relative frequencies, group percentages and photomicrographs.

Results

A total number of 33,506 deliveries were recorded in the hospital during the study period, while one hundred and twenty cases of HM were histologically diagnosed during the study period. No single case of invasive mole was seen. Out of these 120 cases, only 100 met the inclusion criteria and constituted the materials used for this study. CHM [Figure 1] was the most common histologic subtype seen with an incidence rate of 2.03 per 1000 deliveries.

Out of the one hundred molar pregnancies diagnosed histologically within the study period, there were 57 cases (57%) of CHM, 37 cases (37%) of PHM and 6 cases (6%) of EHM.

The age range of patients with HM seen in this study was between 18 and 50 years. Cases of CHM [Figure 1] were found to occur at a slightly older age compared to PHM [Figure 2 and Table 1]. Out of the 100 cases of HMs seen in this study, 72 cases (72%) were diagnosed in the first trimester of pregnancy, while 28 cases (28%) were diagnosed in the second trimester of pregnancy. None of the HMs was diagnosed in the third trimester [Table 2]. Based on the P57^{kip2} IHC staining pattern, HM cases were finally reclassified into 68 cases of CHM and 32 cases of PHM. Amongst the 16 cases that were reclassified, 2 cases initially diagnosed as complete mole were reclassified as a partial mole and 8 cases initially diagnosed as partial mole were reclassified as a complete mole. Out of the 6 cases of EHMs, 5 were reclassified as complete moles and 1 as a partial mole [Table 3].

Discussion

In this study, the incidence rate of HM is 2.98 per 1000 deliveries. This compares favourably with an incidence rate of 3 per 1000 deliveries that was reported previously in the same institution in the study done by Osamor *et al*. in Ibadan in 2002.[12] CHM accounted for majority of the cases of HM seen in this study, followed by PHM. No single case of the invasive mole was found in this study. However, Dauda *et al*.[3] in Gombe and Obaiagbon and Ugiagbe[13] in Benin reported four cases each of invasive mole in similar studies.

The incidence rates from the Nigerian series generally fall within the range of 1.7 per 1000 deliveries to 6.0 per 1000 deliveries.[3-10,12-17] The variations in incidence rates amongst the different studies done in Nigeria may be due to the fact that these rates are actually institutional frequency rates because most of these studies are hospital-based rather than population-based studies. Some of these studies were also done over a relatively short period of time with a small sample size. The incidence rates of HMs also show wide geographic

Table 1: Age distribution of patients with complete and partial moles

Age (years)	CHM (%)	PHM (%)	Total (%)	χ^2 (df)	*P*
10-19	4 (5.9)	2 (6.25)	6 (6.0)	2.92(4)	0.572
20-29	25 (36.8)	16 (50)	41 (41.0)		
30-39	28 (41.2)	12 (37.5)	40 (40)		
40-49	9 (13.2)	2 (6.25)	11 (11.0)		
50-59	2 (2.9)	0 (0)	2 (2.0)		
Total	68 (100)	32 (100)	100 (100)		

CHM: Complete hydatidiform mole, PHM: Partial hydatidiform mole

Table 2: Gestational age distribution of complete and partial moles

Gestational age	CHM (%)	PHM (%)	Total (%)	χ^2 (df)	*P*
First trimester	48 (70.6)	24 (75)	72 (72)	0.21 (1)	0.647
Second trimester	20 (29.4)	8 (25)	28 (28)		
Third trimester	0	0	0		
Total	68 (100)	32 (100)	100 (100)		

CHM: Complete hydatidiform mole, PHM: Partial hydatidiform mole

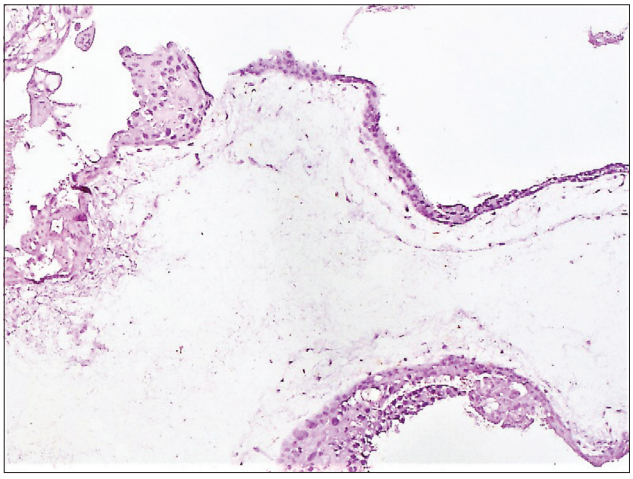

Figure 1: Photomicrograph showing negative nuclear p57^{kip2} immunostain of complete hydatidiform mole with cytotrophoblasts and villous stromal cells. Immunoperoxidase stain (×100)

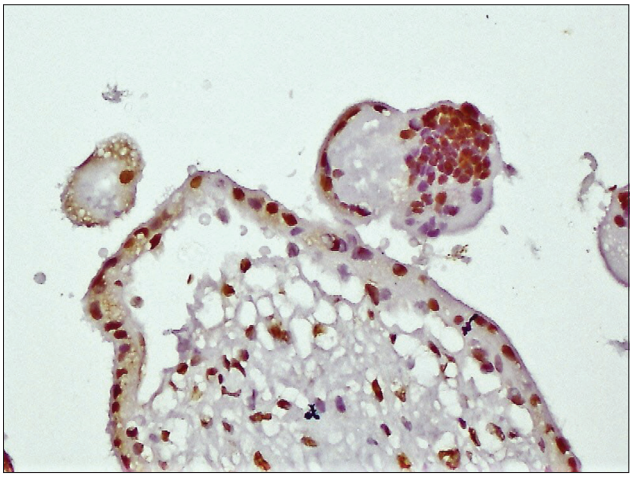

Figure 2: Photomicrograph showing positive nuclear p57^{kip2} immunostain of partial hydatidiform mole with cytotrophoblasts and villous stromal cells. Immunoperoxidase stain (×100)

Table 3: Correlation between morphological diagnosis and final immunohistochemical diagnosis using P57^{kip2}

Histologic diagnosis	Final immunohistochemical diagnosis with P57^{kip2}				
	CHM	PHM	Total	χ^2 (df)	*P*
Complete mole	55 (96.5)	2 (3.5)	57 (100)	58.49 (2)	<0.001
Partial mole	8 (21.6)	9 (78.4)	37 (100)		
Equivocal hydatidiform moles	5 (83.6)	1 (16.7)	6 (100)		
Total	68 (68)	32 (32)	100 (100)		

CHM: Complete hydatidiform mole, PHM: Partial hydatidiform mole

variations globally, with reported estimates ranging from 1 in 500 pregnancies to 1 in 1000 pregnancies in Europe and North America to 1 in 1000 pregnancies to 12 in 1000 pregnancies in some areas of Asia and the Middle East.[18-20] Discrepancies in the use of hospital-based or population-based data can also partly explain these worldwide variations.[20]

The ratio of CHM to PHM found in this study is 2.1:1. The preponderance of CHM as highlighted by this study is comparable to what has been found amongst similar studies across Nigeria and globally.[2,4,5,10,21-24] However, some other studies have reported PHM to be more common than CHM.[13-16] In this study, the age range for HM was 16–50 years, an age range almost similar to what was reported from studies in Ghana and Egypt.[17,22] The majority of the cases of HM were seen in the 20–39 years' age group accounting for 82% of all the cases with a mean age of 30.9 years. Negussie and Belachew in Addis Ababa, Ethiopia, also reported a similar mean age of 30.9 years for HM.[24] This mean age is also fairly close to the 30 years and 31.8 years reported in studies from Maiduguri and Ife, respectively.[5,14] Only 5% of our cases occurred in patients <20 years of age which is far lower than the frequency of 20.6% and 28.2% reported in some studies from Northern Nigeria.[10,14] This may be due to early marriage being more common amongst women from the northern part of the country. In our study, only 13% of the cases occurred in women aged 40 years and above. The relatively lower rate in this age group may also be due to the fact that family planning rates are becoming higher due to increased awareness and better education such that fewer women are getting pregnant at an advanced age.

In the index study, there is a high correlation between the morphologic diagnosis of HM and IHC diagnosis using P57^{kip2}. The maternal decidual cells in the study showed a 100% positive nuclear expression of P57^{kip2}, while the intermediate trophoblasts showed a 97% positive nuclear expression of P57^{kip2}. Jun *et al*. and Castrillon *et al*. also demonstrated a positive expression of p57^{kip2} in the intermediate trophoblasts which are consistent with our findings.[11,25] However, Chilosi *et al*. reported a completely negative expression of p57^{kip2} in the intermediate trophoblasts.[26]

This study like many others did not record any divergent or discordant staining pattern of p57^{kip2}. Besides the rarity of these findings, our small sample size could probably have also limited the observation of such phenomenon. It is thus possible that a larger multi-institutional study in the future combining p57^{kip2} IHC marker with DNA genotyping may show such findings.

Some studies, however, reported these aberrant p57^{kip2} expression patterns.[27,28] This divergent staining pattern of p57^{kip2} IHC marker has been suggested to be due to a twin gestation comprised of a p57^{kip2}-negative androgenetic diploid complete HM and a p57^{kip2}-positive biparental diploid non-molar specimen.[27,28] It could also suggest a mosaic specimen comprised of a p57^{kip2}-discordant non-molar specimen component and a p57^{kip2}-negative complete HM component.[27,28] A discordant p57^{kip2} expression may be due to androgenetic/biparental mosaic/chimeric conceptions.[27-29]

Based on the outcome of this study, P57^{kip2} appears to be a reliable IHC marker for differentiating CHM from a PHM, a finding that is consistent with what other similar studies have reported.[11,25,26,30-33]

Conclusion

P57^{kip2} could be useful as an ancillary histopathologic investigation for differentiating CHM from PHM, particularly in difficult and challenging cases.

Limitation

The study was a hospital-based one and may not adequately and accurately represent the true incidence of HMs in the Southwestern Nigerian population.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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