VALIDATION OF THE HISTOLOGICAL DIAGNOSIS OF HYDATIDIFORM MOLES WITH P57^{KIP2} IMMUNOPHENOTYPING AT THE UNIVERSITY TEACHING HOSPITALS OF KIGALI AND BUTARE (CHUK, CHUB)

Thomas Habanabakize, MD

Master of Medicine (Anatomical Pathology) Dissertation

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By

Thomas Habanabakize, MD

A Dissertation Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Medicine (Anatomical Pathology) of the University of Rwanda.

Supervisor: Dr. Belson Rugwizangoga

Co-supervisor: Dr. Annette Uwineza

University of Rwanda

August, 2021

CERTIFICATION FOR AWARD

The undersigned certify that they have read and hereby recommend for acceptance by the University of Rwanda a dissertation entitled "**Validation of the histological diagnosis of hydatidiform moles with p57^{KIP2} immunophenotyping at the University Teaching Hospitals of Kigali and Butare (CHUK, CHUB)**" in partial fulfillment of the requirements for the Degree of Master of Medicine (Anatomical Pathology) of the University of Rwanda.

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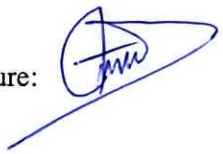
(Co-supervisor)

Date: 2021-12-17

DECLARATION AND COPYRIGHT

I, Thomas Habanabakize, declare that this dissertation is my own original work except where specifically acknowledged and it has not been presented to any other University for similar or any other degree award.

Signature: 

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DEDICATION

I dedicate this dissertation to my family and my friends.

To my wife Brigitte UWINGENEYE together with my daughter Impano Merveille Alda.

ABSTRACT

Background: The hydatidiform moles remain prevalent in spectrum of gestational trophoblastic diseases (GTDs). In resource-limited settings like Rwanda, their definitive diagnosis relies upon single of histomophological diagnosis in addition to clinical and ultrasonography features. The histomorphology alone was found to have interobserver and intra-observer variability and poor diagnostic reproducibility. The present study aimed at determining the role of histological diagnosis of hydatidiform moles and its validation with p57 immunophenotyping.

Methods: This was retrospective observational study embarked at two university teaching hospitals of Kigali and Butare (CHUK, CHUB). Enrolled were all cases of child-bearing women that underwent dilation and curettage or hysterectomy for molar pregnancy between January 2017 and June 2020 and whom histopathological diagnosis was rendered. A review of Hematoxylin &Eosin(H&E) stained slides was performed with subsequent p57 immunostaining after appropriate selection of formalin-fixed paraffin-embedded block (FFPE).

Results: Two hundred eleven (211) cases of hydatidiform moles were recorded over three years and six months' period and 96 (45.9%) cases were all subjected to p57 immunostaining hereby considered as gold standard diagnostic procedure in the diagnosis of hydatidiform moles. As result, the sensitivity and specificity of the histomophological diagnosis of complete hydatidiform mole were estimated at 62.5% and 57.1% respectively with positive and negative likelihood ratio of 0.145 and of 0.54 respectively. Positive and negative predictive value were calculated at 81.8% and 29.3%, respectively. For partial hydatidiform mole sensitivity and specificity of histomophological diagnosis was established at 57.1% and 79.2% whilst positive and negative predictive value counted for 42.9% and 83.8% respectively. The Youden J statistics method was used for accuracy estimation of histomophological diagnosis of hydatidiform mole (HM) and that was 0.196 and 0.336 for both complete and partial hydatidiform moles, respectively. The complete hydatidiform moles were more likely to progress into gestational trophoblastic neoplasia (GTN) as opposed to partial hydatidiform mole (PHM).

Conclusion: This study highlighted a need to integrate p57 immunostaining in routine histopathological diagnosis of hydatidiform moles refining the diagnosis of hydatidiform mole.

Key words: *hydatidiform mole; histology; p57^{KIP2} immunohistochemistry; histomorphology; Rwanda*

LIST OF ABBREVIATIONS

CC: Choriocarcinoma

CHM: Complete hydatidiform mole

CHUB: University of Teaching of Butare

CHUK: University Teaching Hospital of Kigali

DNA: Deoxyribonucleic Acid

FFPE: formalin - fixed paraffin - embedded

GTD: Gestational trophoblastic disease

GTN: Gestational trophoblastic neoplasia

H&E: Haematoxylin and Eosin

HM: Hydatidiform mole

IHC: Immunohistochemistry

IRB: Institutional Review Board

PAb: Primary Antibody

PHM: Partial Hydatidiform mole

SNP: Single-nucleotide polymorphism

STR: Short Tandem Repeats

UR: University of Rwanda

β-hCG: beta-human chorionic gonadotropin

DEFINITIONS

Hydatidiform mole also named molar pregnancy is a part of the spectrum of gestational trophoblastic diseases originating from the placenta with potential to invade the uterus and metastasize.

Triploidy refers to a complete extra set of haploid chromosomes derived from the mother (digynic) or the father (diandric).

Digynic triploidy can result from fertilization of a diploid ovum due to an error at either the first or second meiotic division.

Diandric triploidy may occur through a fertilization of a normal ovum by a diploid sperm or by two sperm (dispermy/double fertilization) and is more common than digynic triploidy (90 versus 10 percent).

Gestational trophoblastic neoplasia refers to malignant transformation of gestational trophoblastic disease including gestational choriocarcinoma, placental site trophoblastic tumour (PSTT) and epithelioid trophoblastic tumour (ETT).

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CHAPTER 1. INTRODUCTION

1.1 Background

Molar pregnancy also named hydatidiform mole, is an abnormal human pregnancy arising from imbalance in or excess of paternal genetic material versus the maternal one resulting in abnormal embryonic development. Therefore, complete hydatidiform moles (CHM) develop following the loss of genetic material from the oocyte, which is then fertilized by two sperm cells or one sperm cell that reproduces its chromosomes. The CHMs (androgenetic diploid ;monospermic-85% being the most common and dispermic androgeny-15%) consisting of only paternal DNA and are most commonly diploid with a 46XX karyotype (but 46XY also occurs) (1). Partial hydatidiform moles (PHM), which are diandric triploid with 69XXY or 69XYY (most common is dispermic of 98%, monospermic-2%) (2) developing secondary to fertilization of an oocyte by two sperm cells resulting in triploidy with a 2:1 paternal to maternal DNA content. Besides, reported are rare familial biparental hydatidiform moles explicited through the demonstration of *NLRP7* or *KHDC3L genes* mutations sharing common imprinting alteration involved in the final development of two specific types of hydatidiform moles (3)(4).

Histologically, both complete and partial hydatidiform moles exhibit hydropic degeneration of chorionic villi and somewhat exuberant trophoblastic cell proliferation. The defining histological features of each of two entities are quite different in most instances. Hence, complete hydatidiform displays diffuse hydropic villi along with circumferential trophoblastic hyperplasia and no fetal tissue whereas partial hydatidiform mole exhibiting partial trophoblastic proliferation along scalloped variably-sized villi with presence of fetal tissues.

While, the histomophological analysis remains the basis for the diagnosis of hydatidiform mole in limited-resources settings inclusive of our country in addition to the molar pregnancies being nowadays evacuated earlier in the first trimester, the diagnosis and the classification have become a challenge over days following on the top of the lack of well-established classic morphological features. Further, the histomorphology alone suffers intra and inter-observer variability along poor diagnostic reproducibility (5).

Moreover, the distinction of PHMs and CHMs from abnormal non-molar villous lesions (NMs) is very crucial for evidenced-based clinical management as well as close follow-up with serum beta human chorionic gonadotropin (β hCG) levels monitoring together with contraceptive use for an earlier detection of possible persistent disease such as gestational trophoblastic neoplasia (GTN). As point of the fact, the latter two entities have demonstrated potential progression into this GTN (6). However, this special follow up is not required for a diagnosis of the non-molar villous lesions (7).More importantly, the genotyping studies or conventional cytogenetics make a distinction from CHMs, PHMs, and the abnormal non-molar villous lesions (NMs) more specifically in discerning the definitional ploidy status of the three entities including diploidy, diandric triploidy, and biparental diploidy respectively. Besides, the NMs share similar histologic features with PHMs and include hydropic abortus, chromosomal abnormalities, digynic triploid conceptions, and placental mesenchymal dysplasia(8).

Nonetheless, the complete hydatidiform moles (CHM) and partial hydatidiform moles (PHM) can be accurately distinguished from each other using immunohistochemistry that detects $p57^{KIP2}$ in trophoblastic tissue. $p57^{KIP2}$ gene on chromosome 11p15.5 encodes a strong inhibitor of several G1 cyclin/Cyclin dependent kinase complexes and is a negative regulator of cell proliferation. This gene is paternally imprinted and maternally expressed, and the presence of its protein product serves as a surrogate marker for the nuclear maternal genome (9)(10).The $p57^{KIP2}$ is an antibody that stains gestational tissue which has the maternal genome. Therefore, PHMs and normal trophoblastic tissues are positive to $p57^{KIP2}$ because they both have maternal and paternal genome. Therefore, $p57^{KIP2}$ can help in identifying CHM but not distinguish PHMs from normal trophoblastic tissues (11).A number of studies reported a perfect interobserver agreement of high sensitivity and specificity of p57 immunochemical staining when compared with genotyping tests such as PCR short tandem repeat. For instance, one recent study reported a sensitivity of 93% to 96% for individual pathologist and 96% by consensus of two gynecologic pathologists whereas a specificity ranging from 96% to 98% for individual pathologist in diagnosing both CHM and PHM respectively though the latter being problematic when it comes to distinguish it from non-molar lesions (NMs) (12).

1.2 Problem statement and justification of the study

The accurate diagnosis of molar pregnancy is essential for both clinical follow-up and management of patients. However, in resource-limited settings like in our country Rwanda, only histopathogical diagnosis is rendered on hydatidiform moles although it is known to have considerable rates of inter/intra-observer variability and poor diagnostic reproducibility.

Moreover, with readily usage of ultrasound as first diagnostic modality, molar pregnancies are nowadays evacuated earlier, posing again a difficult diagnostic challenge with histomorphology alone. Together, these situations show that there is always a chance of misclassification of hydatidiform mole upon the single morphological diagnosis raising up to 20-30% (6) in addition to near-miss diagnoses of clinical moles cases.

In resources constrained settings whereby cytogenetics studies are not available, the use of immunohistochemistry with $p57^{KIP2}$ may be a more affordable and best alternative in distinguishing both morphological types of hydatidiform moles mostly complete types from its mimics. Besides, the conventional karyotyping, which is also available in our clinical settings but not yet accessible for non-blood samples, can be utilized in definitive diagnosis of hydatidiform moles and it may be used for cases in which the IHC did not find to be CHMs. If these tests are performed as package, they will help in real classification, accurate risk stratification and proper prevention of potential malignancies such as choriocarcinoma which has high mortality rate (13) (14).

In this context, a number of studies have pointed out the usefulness of p57 expression which is mostly keeping with the results of universal gold standard test i.e. PCR short tandem repeats. Therefore, the latter serves as a reliable marker for diagnosis of complete hydatidiform moles, and identifying androgenetic cell lines in mosaic conception(2). Aside,p57 may be utilized in discerning all those cases of early first-trimester hydropic placentas and it has demonstrated concordant results with microsatellite DNA genotyping analysis in the latter cases (15).This study paved the way to refine our routine histological diagnosis of hydatidiform moles by $p57^{KIP2}$ immunohistochemistry while conventional karyotyping which are readily available in our clinical settings is to be exploited in coming age for establishment of algorithmic diagnosis of hydatidiform moles.

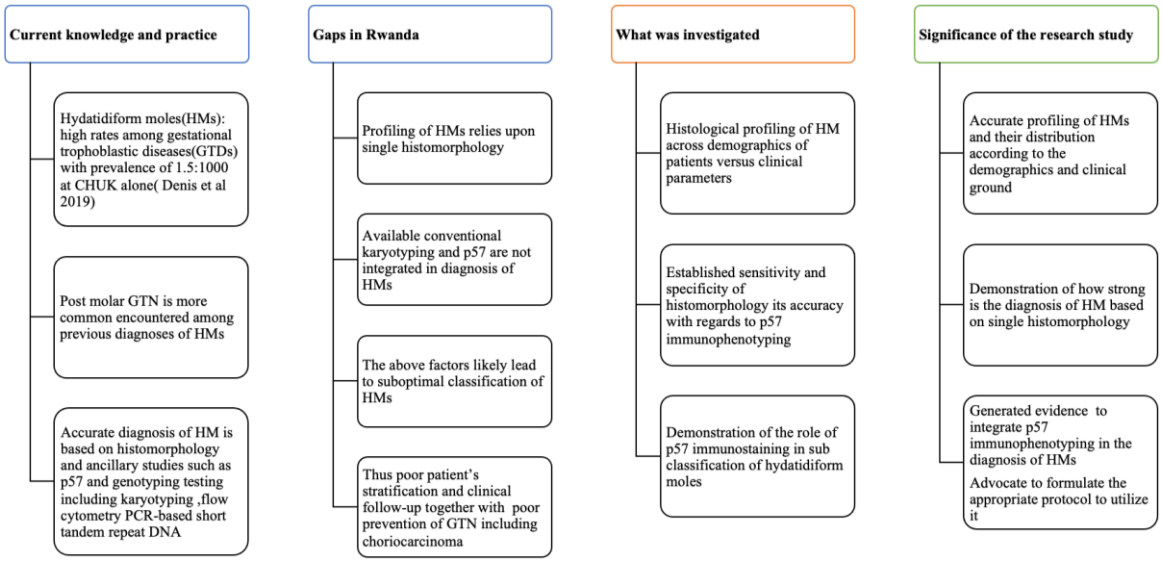

Figure 1. Conceptual framework of the research and its significance to the present research study

This figure summarises the current knowledge and standard practice in the diagnosis of HMs; then gaps in that field in Rwanda are highlighted, from which we show what has been investigated and the importance of performing such investigations, for the betterment of the population of Rwanda.

1.3 Research questions

The present study aimed at answering the following questions:

1) What is the histological profile of hydatidiform moles at two university Teaching Hospitals of Kigali and Butare (CHUK, CHUB)?
2) What is the clinical outcome of hydatidiform moles at the university teaching hospitals of Kigali and Butare (CHUK, CHUB)?
3) What is the sensitivity and specificity of histomorphology with regard to $p57^{KIP2}$immunophenotyping in the diagnosis and classification of hydatidiform moles?

1.4 Objectives

In order to improve the histomorphology of hydatidiform moles and better follow-up of patients to monitor and prevent the occurrence of choriocarcinoma, this study utilized the p57 immunohistochemistry, for the promotion of evidence-based medicine, in order:

3.5.1 General objective

To validate the histological diagnosis of hydatidiform moles with p57 immunophenotyping at university teaching hospitals of Kigali and Butare (CHUK, CHUB)

3.5.2 Specific objectives

1) To determine the histological profile of hydatidiform moles at the university teachings of Kigali and Butare (CHUK, CHUB)

2) To determine the clinical outcome of hydatidiform moles at the university teaching hospitals of Kigali and Butare (CHUK, CHUB);

3) To determine the levels of sensitivity and specificity of histomorphology with regard to $p57^{KIP2}$immunophenotyping in the diagnosis and classification of hydatidiform moles

CHAPTER 2. LITERATURE REVIEW

Worldwide, it is still difficult to establish the incidence or prevalence of hydatidiform moles due to the very low frequency of this group of the diseases along with important variation in reporting the cases across the regions. In Europe the incidence of hydatidiform moles extends from 0.98/1000 to 2.17/1000 deliveries in most the countries (16) while in other parts of the world, Taiwan records highest incidence of 1/125 live births, Japan and South East Asia recording 2/1000 pregnancies) and 1/1500 in United States (4).

In Africa, the values of incidence and prevalence of hydatidiform are presumed to be high with example of recent incidence of molar pregnancy reported to be 13.1 and 3.2 per 1000 live births in lower Egypt(4).In Rwanda, single study conducted by Rwabizi et al revealed a prevalence of Gestational trophoblastic diseases(GTDs) of 1.5/1000 live birth mostly comprising complete and partial hydatidiform moles(17).

Further, hydatidiform moles remain prevalent amongst gestational trophoblastic diseases and reported at 97.40% (18) in recent studies whereas the overall occurrence is still reported to be 23 to 1299 per 100,000 pregnancies.

Hydatidiform moles (HMs) are premalignant diseases with potential to transformation into gestational trophoblastic neoplasia (GTN). The neoplastic transformation into GTN is at 15-30% for complete hydatidiform moles and around 1-7% for partial hydatidiform moles (19).Gestational trophoblastic neoplasia (GTNs) comprise less commonly placental site trophoblastic tumor, epithelioid trophoblastic tumor, invasive mole and choriocarcinoma. The latter being a frank malignant epithelial neoplasm that develops in 1/40 hydatidiform moles and more frequently in cases of CHMs with risk of 3-5% against PHM with rare risk of transformation(20). The prevalence of choriocarcinoma (CC) is reported to occur in around 10:50,000 deliveries in one study (21)and its incidence has been increasing over the last 30years(22). The same trend of increase in the incidence of GTD including mostly hydatidiform moles was observed in a previous study at CHUK, with an occurrence of 1.5:1000 deliveries(17). It is therefore important to detect and subclassify the hydatidiform moles, as way to monitor and prevent the occurrence of gestational choriocarcinoma, a pure epithelial malignancy. When

looked at in any pregnancy events, 50% GTN develop following hydatidiform moles, 25% from miscarriage or tubal pregnancy and 25% from a normally occurred pregnancy(13).

It has been demonstrated that a tissue DNA genotyping, for example PCR short tandem repeats is such a feasible and highly accurate method for the confirmation and subtyping hydatidiform moles(23). Additionally, conventional cytogenetics seems to be more reliable predictor of risk of transformation into gestational trophoblastic neoplasia(GTN) and more informative in classifying the hydatidiform moles than histomorphology. Nonetheless, immunophenotyping using $p57^{KIP2}$ can be routinely used to distinguish CMHS from PHMs and other types of lesions in trophoblastic tissue on one hand, while on the other hand, conventional karyotyping would be used in clinical settings to distinguish PHMs from other non-molar lesions of the trophoblastic tissue.

Like in any other resource-limited settings, the use of obstetrical ultrasound in the evaluation of pregnancy-associated bleeding versus accurate testing for beta human chorionic gonadotropin (β-hCG) levels constitute one of our standard care-based diagnosis of molar pregnancy. Of note, these basic tools are followed by histopathological analysis of the tissue although the latter diagnostic package remain limited to the tertiary hospitals. Additionally, in high-resourced settings the definitive diagnosis of molar relies upon to both clinic-pathological features and ancillary-tests including immunohistochemistry, ploidy studies (cytogenetics, flow cytometry) and DNA genotyping (PCR short tandem repeats).

Therefore, the present study aimed at refining the histological diagnosis of hydatidiform moles shedding lights to improved clinical follow up as well as evidencing the current histological profile of hydatidiform moles at University Teaching Hospitals of Kigali and Butare (CHUK, CHUB).

CHAPTER 3. RESEARCH AND METHODS

3.1 Study design and period

This was a retrospective observational study carried out over a period of three years and six months (January 2017 through June 2020)

3.2 Study sites

The study was embarked at two university teaching hospitals of Kigali and Butare (CHUK, CHUB) in Anatomical Pathology units.

3.3 Study population

All women with clinical features of molar pregnancy and with confirmed histopathological diagnosis of hydatidiform mole were enrolled in the study.

3.4 The primary outcomes

Independent variable: histological data, demographic data (age, residency), clinical data (ultrasound findings, gravidity, gestational age, pre-treatment and follow up beta hCG levels)
Dependent variables: $P57^{KIP2}$ immunostaining pattern, clinical outcome

3.5 Study sample selection

3.5.1 Inclusion criteria

All clinically suspected cases of hydatidiform moles (i.e., clinical history and physical examination, ultrasound findings and beta hCG levels) were included for histopathological diagnosis and reviewed with subsequent validation by $P57^{KIP2}$ immunostaining.

3.5.2 Exclusion criteria

All Cases of non-molar lesions by histomorphology were excluded from the study.

3.6 Sample size

The sample size was calculated using the formula as follows for cross-sectional studies:

$$\boldsymbol{n = \frac{Z^2 \times P \times Q}{d^2}}$$

n= the sample size, Z= the normal deviation P= the expected proportion, Q=l-P

d= required precision.

The expected proportion (P) of 6.1% the established prevalence of hydatidiform mole in our region (24)since there is no known prevalence rate of hydatidiform mole in our settings. As P values are considered significant when below 5%, hence Z= 1.96 was used in this formula with precision of 5%, hence (d=0.05).

Sample size;

$$n = \frac{(1.96)^2 \times 0.061 \times (1 - 0.061)}{(0.05)^2} = 88$$

Sample size for comparing the sensitivity (or specificity) of two diagnostic tests in diagnostic study(25)

$$n = \frac{\left[z_{\frac{\propto}{2}}\sqrt{2 \times \bar{P}(1-\bar{P})} + Z_\beta\sqrt{P_1(1-P_1) + P_2(1-P_2)}\right]^2}{(P_1 - P_2)^2}$$

According to the above formula, almost the same sample size is calculated as follows:

P:96%, Z_β: 0.84 Zα: 1.96, P1 = 0.96, P2 = 81% and P ¼ 0:75, then

$$\mathbf{n} = \frac{\left[\mathbf{1.96}\sqrt{\mathbf{2 \times 0.88 \times 0.12}} + \mathbf{0.84}\sqrt{\mathbf{0.96(0.04) + 0.81(0.19)}}\right]^2}{\mathbf{(0.15)^2}} = \mathbf{92}$$

3.7 Study procedure

For cases enrollment, the slides were reviewed for histological diagnosis of hydatidiform moles followed appropriate selection of FFPE block with subsequent immunostaining with $p57^{KIP2}$.

Formalin fixed paraffin embedded blocks (FFPE) were obtained from archive for $P57^{KIP2}$ immunostaining. A normal placenta has been collected for external positive control of $p57^{KIP2}$

immunostaining together with maternal decidua and intermediate or extra/trophoblastic cells at the site of implantation that served as internal positive control.

The data collection sheet comprising patient clinical data, histomophological diagnosis, p57 IHC, clinical outcome and clinical follow-up time was pre-designed for this present study.

$p57^{KIP2}$Immunohistochemistry and interpretation

In the present study, 96 cases corresponding formalin-fixed, paraffin-embedded blocks depicting CHM, PHM, or HM, unspecified type were selected from the archives in the Department of Pathology, Anatomical Pathology unit at two university hospitals of Kigali and Butare (CHUK, CHUB) between January 2017 and June 2020. The original identification of CHM, PHM, and HM, unspecified type cases was based on previous histologic evaluation of H&E-stained sections from either product of dilation and evacuation or hysterectomy specimens.

Immunohistochemical staining with anti-$p57^{KIP2}$ mouse monoclonal antibody (clone KP10, 25% dilutions of 1.11µg/Ml pure dose) was operated using an avidin-biotin immunoperoxidase complex (ABC) method (detailed full protocol is found in appendices of this dissertation). The immunostaining result was interpreted as positive for p57 with similar staining pattern in either decidual cells and/or extravillous trophoblastic cells, which served as an internal positive control and exhibited positive nuclear $p57^{KIP2}$ staining in both villous stromal cells and/or cytotrophoblasts. P57 was negative when there was lack of p57 expression in both villus cytotrophoblasts and villous stromal cells. The scoring of nuclear positivity and negativity for p57 IHC was established according to the study conducted by Karthi. P. Kumar, and P. S. Jayalakshmy (26) as follows: 0,1+ were interpreted as negative for p57 (no nuclear staining and 1-10% of positive cells) whereas 2+, 3+ (10-50% positive cells and >50% positive cells respectively) were considered as positive expression.

Of note, independent pathologist and I were blinded for previous histomophological diagnosis and signed out corresponding p57 immunostained slides for definitive recording of the diagnosis in the present study.

3.8 Enrollment and data collection

During the period of this study, we retrieved archived H&E slides of hydatidiform moles together with recorded information on the request form and in OPENCLINIC at both university teaching hospitals of Kigali and Butare (CHUK, CHUB). Demographic data (age, residency), clinical data (ultrasound findings, gravidity and gestational age, β-hCG levels prior to treatment and post-follow up levels) were recorded and 96 cases immunostained with p57 for which the FFPE Blocks were available.

3.9 Data management and statistical analysis

A structured questionnaire served as main tool of data entry and together with Excel spreadsheet with password and the analysis performed using Statistical analyses were performed using Statistical Product and Service Solutions (SPSS) version 25 (IBM Corporation, New York 10504-1722, USA) and MedCalc (MedCalc Software, Mariakerke, Belgium) v.10.2.0.0 from which the descriptive statistics were obtained including frequencies, percentiles. Also, computed were sensitivity, specificity, negative and positive likelihood ratios, negative and positive predictive values, odds ratio with confidence interval of 95%.

In this portion of the research p57 IHC was considered as gold standard to assess the histomophological diagnostic modality and its accuracy by Youden J statistics method. The relationship between categorical variables were established using Chi-square and Chi-square for trend. P values were considered statistically significant when $p < 0.05$.

3.10 Ethical considerations

3.10.1 Confidentiality

There were no risks to patient since this research did not engage the subjects, only operated in the laboratory settings whereby a retrieval of H&E slides for histological review and then appropriate FFPE block selection for $P57^{KIP2}$ immunostaining. Every single case was assigned a research code corresponding to histopathology lab number and no identification appeared on data collection sheet. Besides, data were entered into password-protected excel spread sheet along with secured SPSS 25 version for analysis.

3.10.2 Ethical approval

The Anatomical Pathology program of College of Medicine and Health Sciences (CMHS) at the University of Rwanda (UR) issued a scientist approval to our study, submitted into University Institutional review board (IRB) that granted its ethical approval (No 081/CMHS IRB/2020) and thereafter authorization letters (RC/UTHB/008/2020 and EC/CHUK/0134/2019) to conduct the study at two teaching hospitals were obtained from respective Research ethics committees of the above hospitals.

3.11 Strength, problems and limitation of the study

3.11.1 Strength of the study

This was the first study of its kind in Rwanda to have generated pilot data on histological profile of hydatidiform moles with validation by p57 immunophenotyping. It has demonstrated a need to integrate p57 immunostaining in proper stratification of hydatidiform for evidenced-based treatment and clinical follow-up of affected patients.

3.11.2 Problems and limitations of the study

Being retrospective study in nature, we had incomplete data for evaluation of both clinical outcome and follow-up of all histologically diagnosed cases. It would have been useful to perform cytogenetics as gold standard diagnostic modality of hydatidiform moles but we did not do it due to financial constraints though these ones were pending from the university of Rwanda that postponed the grant.

CHAPTER 4. RESULTS

Over a period of three years and half, we recorded 211 cases of HM and of which Ninety-six (96) cases were subjected to p57 immunohistochemically staining at two teaching university hospitals, as shown in Table 1. The median age was 32years old. The histopathology diagnosis of hydatidiform mole was more likely prevalent among women aged between 21 and 40 years old (55.5%) followed by those aged above 40years old representing 42.2%. Complete hydatidiform mole was mostly occurring in this age range followed by partial hydatidiform moles. Most women were gravid one to three (G1-G3) between 11weeks and 20weeks of gestational age range (11W-20W) representing 27.7%. The p57 immunostaining was done on 96 cases and served to stratify five cases unspecified hydatidiform mole by histomorphology as complete hydatidiform mole (see Figure 2 for photomicrographs of the typical staining pattern).

Table 1.Demographics and obstetrical features of the patients

Variables	n	Percentage
Age (n=211, median=32.0; Q1-Q3=28.0 – 43.0) years		
≤20 years	5	2.4
21-40 years	117	55.5
>40 years	89	42.2
Province of origin (n=211)		
Eastern	10	4.7
Western	18	8.5
Northern	25	11.8
Southern	124	58.8
Kigali capital	16	7.6
Not recorded	18	8.5
Gravidity (n=211)		
G1-G3	45	21.3
G4-G6	23	10.9
G7-G10	11	5.2
>G10	7	3.3
Not recorded	125	59.2
Gestational age in weeks (n=211)		
1-10 Weeks	7	3.3
11-20 Weeks	27	12.8
21-30 Weeks	15	7.1
31-40 Weeks	5	2.4
Not recorded	157	74.4
Treatment modalities (n=92)		
Dilation and evacuation	76	79.2
Hysterectomy	20	20.8
Ultrasound findings (n=211)		
Suggestive	60	28.4
Unremarkable	59	28.0
Not recorded	92	43.6
β-hCG (n=211, median=35650; Q1-Q3=8225-195203)		
<1000	8	3.8
1000-200000	41	19.4
>200000	33	15.6
Not recorded	129	61.1
Histomorphology findings (n=211)		
Complete hydatidiform mole	119	56.4
Partial hydatidiform mole	49	23.2
Hydatidiform, unspecified	30	14.2
Invasive hydatidiform mole	13	6.2
P57 Immunophenotyping results (n=96)		
Positive	21	21.9
Negative	72	75.0
Equivocal	3	3.1
Reported clinical outcome (n=211)		
Markedly decreased β-hCG levels and cured	31	14.7
Transformed into invasive hydatidiform	1	0.5
Transformed into choriocarcinoma	1	0.5
Not reported	178	84.4

β-hCG: Beta human chorionic gonadotrophin …; Q1/Q3:Interquartiles1/3

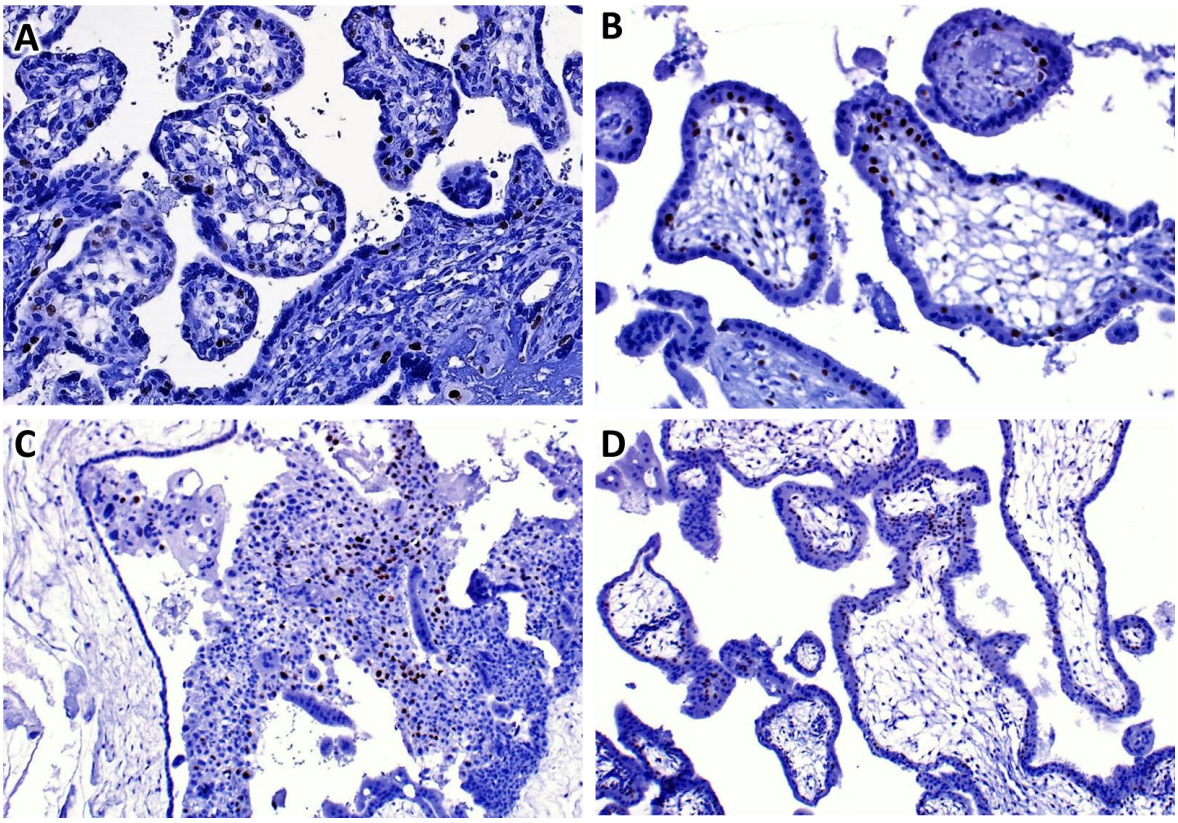

Figure 2 .Photomicrographs of p57 expression across histomophological diagnosis of HM

Photomicrograph C illustrates negative p57immunostaining (intermediate trophoblastic cells and maternal decidua serving as internal positive control); B, D: positive p57immunostaining of both villus cytotrophoblasts and stromal villous cells in keeping with partial hydatidiform mole A: positive external control (placenta)

From this Table 2 below, 96 cases were subjected to p57 immunostaining. Thus, the sensitivity and Specificity of the histomophological diagnosis to diagnose complete hydatidiform mole was 62.50% and 57.10% respectively whereas the positive and negative predictive value estimated at 81.8% and 29.30%. For partial hydatidiform mole, sensitivity and specificity of histomophological diagnosis was established at 57.10% and 79.20% respectively while the values of positive and negative predictive were computed at 42.90% and 83.80% respectively with statistical significance (p=00.4). The accuracy of histomorphology to diagnose complete hydatidiform moles with Youden J statistics method is 19.6% (0.196) whereas for partial hydatidiform mole the accuracy goes up to 36.6% (0.366). Positive and negative likelihood ratios were computed at 1.45 and 0.54 meaning a very small value or rarely useful test alone.

Table 2. Sensitivity, specificity, negative and positive predictive value of histomorphology diagnostic modality with regards to p57 immunostaining taken as gold standard diagnostic test in this study

Type of hydatidiform mole	P57 Immunophenotyping		P value	Sensitivity	Specificity	PPV	NPV
	Positive	**Negative**					
Complete hydatidiform mole							
Yes	9 (16.4%)	**45 (81.8%)**	0.19	62.50%	57.10%	81.80%	29.30%
No	**12 (29.3%)**	27 (65.9%)					
Partial hydatidiform mole							
Yes	**12 (42.9%)**	15 (53.6%)	0.004	57.10%	79.20%	42.90%	83.80%
No	9 (13.2%)	**57 (83.8%)**					

*+LH: Sensitivity/(1-specificity) +LH=0.625/ (1-0.571) =**1.45** (1-1.9) very small/rarely useful test*

*-LH= (1-Sensitivity)/Specificity -LH= (1-0.571)/0.792=**0.54**(0.51-1.0) very small/rarely useful test.*

The table 3 below demonstrates that women above 40 years of age were 2.85 times more likely to have complete histomorphology compared to women of 40 years old and (below OR=2.85; 95% CI: 0.94-8.64; p=0.063). Women with βhCG count >200,000 were 2.64 times more likely to have complete histomorphology as those with βhCG count ≤200,000 (OR=2.64; 95% CI: 0.50-13.8; 0.251). There was no difference in histomorphology according to the ultrasound findings.

Women below 40 years of age were 3.81 times more likely to have positive P57 Immunophenotyping compared to women of above 40 years of age (OR=3.81; 95% CI: 1.02-14.2; p=0.045). Women who had not suggestive ultrasound findings were 1.72 times more likely to have positive P57 Immunophenotyping as those with suggestive ultrasound findings (OR=1.72; 95% CI: 0.32-9.09; 0.637) and women with βhCG count of ≤200,000 were 1.43 times more likely to have positive p57 Immunophenotyping as those with βhCG count of >200,000 (OR=1.43; 95% CI: 0.51-3.96; p=0.493)

Table 3: Association between clinical parameters across both histomophological diagnosis of HM and p57 immunophenotyping

Variables	Histomorphology		OR (95% CI)	P value
	Partial	Complete		
Age				
≤40 years	25	35	2.85 (0.94-8.64)	0.063
>40 years	5	20		
βhCG				
≤200000	25	12	2.64 (0.50-13.8)	0.251
>200000	11	2		
U/S findings				
Not suggestive	33	18	1.20 (0.47-3.08)	0.704
Suggestive	22	10		

Variables	P57 Immunophenotyping		OR (95% CI)	P value
	Positive	Negative		
Age				
≤40 years	18	44	3.81 (1.02-14.2)	0.045
>40 years	3	28		
βhCG				
≤200000	10	32	1.72 (0.32-9.09)	0.637
>200000	2	11		
U/S finding				
Not suggestive	14	42	1.43 (0.51-3.96)	0.493
Suggestive	7	30		

CI: confidence interval; U/S: ultrasound; β hCG: beta human chorionic gonadotrophin; OR; odds ratio

Figure 3 is a diagram illustrating the role of p57 immunostaining in classifying the morphological challenging cases of hydatidiform whereby in our study five cases of unspecified type were classified as complete hydatidiform by p57 and seven cases of invasive hydatidiform moles were stratified as complete type by p57 immunostaining.

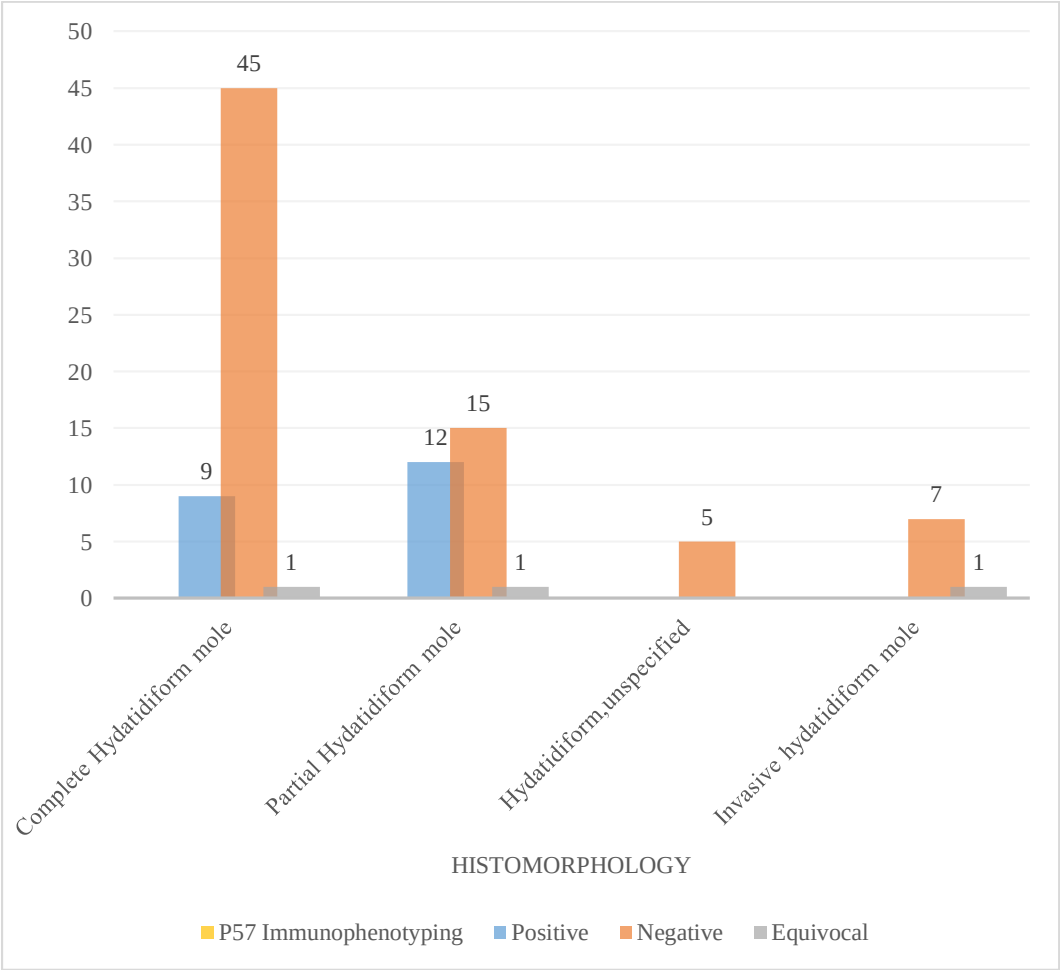

Figure 3 Histogram depicting p57 expression across histomophological diagnosis of hydatidiform moles

CHAPTER 5. DISCUSSION

Hydatidiform moles include a form of abnormal human pregnancy displaying characteristic hydropic chorionic villi along trophoblastic proliferation. It is stipulated that genomic imprinting is implicated in the formation of hydatidiform moles although their pathogenesis remains ununderstood (3).In this study, we aimed at validation of histological diagnosis by immunostaining with anti-p57 monoclonal antibody that is labelling nuclear maternal genome. Besides, we attempted to have a look at clinical outcome and distribution of two histomophological forms of hydatidiform moles.

Histological profile of hydatidiform moles and age at presentation

Over three years and six months period, 211 histological cases of hydatidiform moles were recorded inclusive of complete hydatidiform mole representing 56.4% followed by partial hydatidiform moles (23. 2%). The same distribution of hydatidiform moles was confirmed when 96 cases were subsequently subjected to p57 immunostaining; the complete hydatidiform moles being the predominant type and followed by partial hydatidiform moles. Our findings are supported by what reported by Yassemine Khawajkie *et al.* (1) wherein they even performed more ancillary studies on their cohort cases studies including ploidy studies (Flow cytometry and SNP) and genotyping test such as PCR short tandem repeats (STR).Further, this trends reflects the utility of integrating ancillary tests in proper classification of hydatidiform moles and definitive stratification of patients for best clinical follow-up and management of post-molar gestational neoplasia.

Nonetheless, Nawras Najah Mubarak *et al.* reported different single-based histopathological distribution to what we found as similarly recorded in a number other reports such that partial hydatidiform moles was the most common subtype followed complete hydatidiform moles (27).Although, no highlighted explanation in their report for this different histological profile, it can be justified by utilizing ancillary tests.

The majority of cases were encountered in women aged between 21-40years with median age of 32.0(28.0-43.0) and followed by those aged above 40years against women aged below 20 years in whom the hydatidiform moles were less represented. These findings are in line with the results

obtained by Yassemine Khawajkie *et al.* (204 cases of HM) and they reported almost the same age range of 21-30 versus 31-40 years and those above 40. Thus, androgenetic monospermic and dispermic CHM were the most common subtype of HM (45 (39.4% were in between 21 and 30, 43 (37.7%) were in between 31 and 40, and 20 (17.5%) were older than 40 years of age) concurring with what we found in our study while triploid dispermic PHM represented in the same age range of 21-30,31-40 years of age ,37.6% and 60.9% is higher than what we found.

In our study, we found cases of complete and partial hydatidiform moles above 40years old whereas Yassemine *et al*. did find few cases above 40 when genotyping testing was performed on their study cases and the latter ancillary test would have made such a difference in different numbers of CHM eventually highlighting the role cytogenetics studies in diagnosing the hydatidiform moles. The median age at the diagnosis of HM in our study was 32.0 years, almost similar mean age of 32.5 years reported in the study conducted by Abimbola O. Kolawole *et al.* (28),lower age than what is reported by Yassemine *et al.* of 33 and 36 years, higher than one reported by Ahmed et al of 26.22 years(4) while median age 22 years reported by Madi *et al*. (3)(29).Our obtained age range translates a positive association of hydatidiform moles with increasing maternal age reported in many other studies(1)(24) and complete hydatidiform mole subtype being more represented as reported in other studies (20)(24).

In our present study, women aged above 40 years of age were 2.85 times more likely to have complete mole histomorphology compared to women aged 40 years and below (below OR=2.85; 95% CI: 0.94-8.64; p=0.063). These findings are in agreement with what other authors reported the extreme maternal age being independently associated with hydatidiform moles and explained by possible unnatural fertilization of an oocytes (30)(24) .

Gravidity and gestational age at the diagnosis of hydatidiform moles

Our study showed that most affected women by hydatidiform were multigravida(G1-G3) in their late first trimester through second trimester (11-20WA) and similar findings were reported in other studies (28) (31) and these data also translate a possibility to encounter many cases of early hydatidiform moles which pose a diagnostic difficulty on single histomorphology with eventual of misclassification.

Clinical outcome of hydatidiform moles at two university teachings of Kigali and Butare

Looking at clinical outcome measured by decrease β-hCG levels with median value 35650 m.I.U/mL (8225-195203 m.i.u/ml), we did not find a significant association across different types of hydatidiform moles with regard to their progression to GTN (p= 0.402 with chi-square for trend of 0.6339) and similar findings were reported by Ahmed Zakaria *et al.* in their prospective study(4) whereby mean pre-evacuation β-hCG levels were higher than what we found in our study. Besides, our study showed that complete hydatidiform moles subtypes together with those stratified by p57 IHC were the most likely to progress into post-molar gestational trophoblastic neoplasia as opposed to partial hydatidiform mole subtype. These findings were in line with the same results obtained in other studies(1). Women with β hCG count >200,000 were 1.43 times more likely to have complete mole histomorphology as those with β hCG count ≤200,000 (OR=2.64; 95% CI: 0.50-13.8; 0.251) and this supports the usual higher β hCG levels associated with complete moles types. In addition, p57 immunophenotyping was likely positive in the latter cases and more positive in cases where ultrasound was not suggestive. These findings point out the positivity of p57 in partial hydatidiform cases than complete hydatidiform cases (OR=1.43; 95% CI: 0.51-3.96; p=0.493).

Sensitivity and specificity of histomorphology with regard to $p57^{KIP2}$ immunophenotyping in the diagnosis and classification of hydatidiform moles

The p57 immunostaining served to accurately stratify the cases of hydatidiform moles. In our study, the most common histological subtype was complete hydatidiform mole representing 56.4% what is different from Nawras Najah Mubarak *et al*. that reported partial HM, the most common followed by CHM(27) ,such a trend is explained by the fact the latter is purely descriptive study and the results of histological profile are of single-based histomorphology findings hence recalling for interobserver variability and poor diagnostic reproducibility among pathologists when it comes to histomophological diagnosis of hydatidiform in absence of ancillary tests. Likewise, our findings were reduplicated in the report of Kumar *et al*. (26).

The sensitivity and specificity of histomophological diagnosis were calculated at 62.5% and 57.1% for complete hydatidiform whereas for partial hydatidiform moles, they were estimated at 57.1% and 79.2%.The above estimated levels of sensitivity and specificity concur with those

established in a study conducted by Madi *et al.* 2018 in which they improved values of sensitivity and specificity using p57 that is 59% to 100% of sensitivity for histomorphology of complete hydatidiform mole and 91% to 96% of specificity whilst for partial hydatidiform mole(PHM),the sensitivity 56% to 93% with specificity of 58% to 92% (29). Thus, our results demonstrate gaps to bridge by initiating p57 IHC adjunct the diagnosis of hydatidiform moles as it has been proven to highly sensitive and specific in discerning complete hydatidiform from its mimics in various studies (32). The accuracy of the histomophological diagnosis was low 0.196 and 0.366 for both complete and partial hydatidiform moles respectively. The latter similar low accuracy of single histomophological diagnosis was also highlighted by other studies that quoted interobserver variability and suboptimal reproducibility amongst even experts of the field of gynecologic pathology(15)(33) with a rate of 20-30% of misclassified hydatidiform moles by single-based histomophological alone. Hence, p57 immunostaining would be opted as adjunct to histomorphology of HM at least in settings of challenging cases.

CHAPTER 6. CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Our study established relatively low levels of sensitivity and specificity for histomophological diagnosis of hydatidiform moles at both university teaching hospitals of Kigali and Butare (CHUK, CHUB) when compared to the estimated levels in other high resourced settings. Thus, a need to incorporate p57 immunostaining in diagnosis of hydatidiform moles as way to refine and support the histomophological diagnosis of molar pregnancy in a subset of cases that might pose a diagnostic challenge. The obtained profile highlighted challenges in classification of hydatidiform moles based on single histomorphology as reported in many other studies.

6.2 Recommendations

In line with the findings of the present study findings:

On one hand it should be recommended to the obstetricians and gynecologists on close follow-up of affected women with hydatidiform moles along with documented βhCG levels together with their appropriate stratification.

On the other hand, to the pathologists, it would also be recommended to properly subclassify the hydatidiform moles into complete and partial categories by both histomorphology and ancillary studies. That would be the best way to refine the histological diagnosis and evidence-based clinical follow up of the patients.

To the policy makers, they are called upon to include at least p57 IHC among other purchased reagents as it can be utilized in reserved settings of morphologically challenging cases of complete hydatidiform mole from its mimics. This is mainly justified by other reported findings in line to what we found in which clinical parameters such as ultrasonography, clinical data and beta human chorionic gonadotrophin (βhCG) levels do not suffice to render the definitive diagnosis.

Further prospective studies are recommended to establish incidence of hydatidiform as well as providing evidence of cytogenetics studies utility including conventional karyotyping that is available in our settings and not yet exploited in this specific context.

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APPENDICES

Protocol for p57 immunohistochemistry(adapted)

Immunostainer

- ✓ Type: Manual

Primary antibody

- ✓ Clone : p57 (Kp10) PAb, Cell Marque
- ✓ Producer: ROCHE DIAGNOSTICS
- ✓ Product no. / lot no.: 60-4617
- ✓ Diluent: Antibody Diluent
- ✓ Dilution factor: 1:4(25%)

PRINCIPLE:

The presence of an antigen was demonstrated using the avidin-biotin peroxidase method of immunohistochemistry.

Specimen: Formalin-fixed, paraffin-embedded tissue sections were cut on a microtome at

5 microns thick and picked on charged slides (IHC slides).

Materials:

- o Oven set at 60 degrees Celsius
- o Water bath set at 80 degrees
- o Gloves
- o Pipettes
- o Xylene
- o Ethanol
- o Tris- buffered saline (TRIS), pH 7.6
- o Envision Flex target retrieval solution (50x) concentrated
- o Envision Flex kit for detection
- o Gill's hematoxylin
- o Resin mountant

- Controls: -positive and negative controls

PROCEDURE

After deparaffinization and hydration to buffer, tissue sections were subjected to heat –induces epitope retrieval (HIER) as follows:

1. Prepared a working solution by diluting the Envision Flex Target Retrieval solution (50x) concentrate 1:50 distilled or deionized water
2. Placed staining jars containing retrieval solution in water bath
3. Heated water bath and jars to 97 degrees. Cover jars with lids to stabilize the temperature and avoid evaporation
4. Immersed sections in the preheated Envison Flex Target Retrieval solution (working solution) in the staining jars.
5. Brought temperature of the water bath and Envision Flex Target Retrieval solution back to 97 degrees celicius incubate for 20 minutes at 97 degrees Celsius
6. Removed the entire jar with slides from the water bath. Allow slides to cool in the Envision Flex Target Retrieval solution for 20 minutes at room temperature
7. Decanted the envision flex target retrieval solution and rinse sections with diluted room temperature envision flex wash buffer for I -5 minutes.

STAINING PROCEDURE USING HUMIDITY CHAMBER

a) Removed the excess of wash buffer from around the sections and applied Blocking reagent “Flex peroxidase” for 5-8 min in humidity chamber at room temperature
b) Rinsed in wash buffer for 4 changes 2 min each
c) Removed the excess of wash buffer from around the sections (both controls and samples on separate slides) and apply primary antibody for 40 min at room temperature in humidity chamber
d) Rinsed in wash buffer for 4 changes 2 min each
e) Removed the excess of wash buffer from around the sections and apply ready to use second antibody “FLEX/ HRP: horse radish peroxidase” for 25-30 min in humidity chamber
f) Rinsed in wash buffer for 4 changes 2 min each

g) Removed the excess of wash buffer from around the sections and apply chromogen: freshly diluted: prepared Flex DAB in substrate buffer (2drops / 1ml); incubated 5 min in humidity chamber

h) Washed in smoothly running tap water for 5 min

i) Count stained in Gill's hematoxylin 30- 45 sec

j) Bluing in running water for 5 min

k) Dehydrated in absolute alcohol, two changes, 2 min each

l) Clearing in xylene two changes, 2 min each

m) Mounted in permanent medium

INTERPRETATION OF RESULTS

The di-amino-benzidine containing substrate solution gave a brown color at the site of the target antigen recognized by the p57(K10) PAb. The brown color was present in both normal placentae that served as external nuclear positive control and decidua cells and intermediate cytotrophoblasts (internal positive control) syncytiotrophoblasts served as internal negative control.

Data Collection form

Form number: ☐

Research code: ☐

Histopathology number: ☐

Demographic data

1. Age: ☐

2. Residence**:** Kigali City ☐ East ☐ West ☐ North ☐ South ☐

Others ☐

Clinical data

1. Type of specimen: D&E ☐ Hysterectomy ☐

2. Obstetrical data: Gestational formula (Gravidity, Parity) ☐ Gestational age ☐

β-hCG levels ☐ Ultrasound findings: suggestive ☐ normal ☐

Histopathology result and p57immunostaining pattern

1. Complete hydatidiform mole ☐ Partial hydatidiform mole ☐

Others ☐

2. $P57^{KIP2}$expression: Positive ☐ Negative ☐ equivocal

Clinical outcome and time for follow-up

Reduced β-hCG levels and cured ☐

Transformed into gestational trophoblastic neoplasia(GTN) Yes ☐ No ☐

Time for follow up : 1-3months ☐ 4-6months ☐ more than 6 months

CMHS INSTITUTIONAL REVIEW BOARD (IRB)

Kigali, 20th May 2020

Dr HABANABAKIZE Thomas
School of Medicine and Pharmacy, CMHS, UR

Approval Notice: No 081/CMHS IRB/2020

Your Project Title ***"Validation of the Histological Profile of Hydatidiform Moles Using P57KIP2 Immunophenotyping and Conventional Karyotyping at the University Teaching Hospital Of Kigali (CHUK) And Butare (CHUB)"*** has been evaluated by CMHS Institutional Review Board.

		Involved in the decision		
			No (Reason)	
Name of Members	**Institute**	Yes	**Absent**	**Withdrawn from the proceeding**
Prof Kato J. Njunwa	UR-CMHS		X	
Prof Jean Bosco Gahutu	UR-CMHS	X		
Dr Brenda Asiimwe-Kateera	UR-CMHS	X		
Prof Ntaganira Joseph	UR-CMHS	X		
Dr Tumusiime K. David	UR-CMHS	X		
Dr Kayonga N. Egide	UR-CMHS	X		
Mr Kanyoni Maurice	UR-CMHS		X	
Prof Munyanshongore Cyprien	UR-CMHS	X		
Mrs Ruzindana Landrine	Kicukiro district		X	
Dr Gishoma Darius	UR-CMHS	X		
Dr Donatilla Mukamana	UR-CMHS			
Prof Kyamanywa Patrick	UR-CMHS		X	
Prof Condo Umutesi Jeannine	UR-CMHS		X	
Dr Nyirazinyoye Laetitia	UR-CMHS	X		
Dr Nkeramihigo Emmanuel	UR-CMHS		X	
Sr Maliboli Marie Josee	CHUK	X		
Dr Mudenge Charles	Centre Psycho-Social	X		

After reviewing your protocol during the IRB meeting of where quorum was met and revisions made on the advice of the CMHS IRB submitted on 20th May 2020, **Approval has been granted to your study.**

Please note that approval of the protocol and consent form is valid for **12 months**.

You are responsible for fulfilling the following requirements:

1. Changes, amendments, and addenda to the protocol or consent form must be submitted to the committee for review and approval, prior to activation of the changes.
2. Only approved consent forms are to be used in the enrolment of participants.
3. All consent forms signed by subjects should be retained on file. The IRB may conduct audits of all study records, and consent documentation may be part of such audits.
4. A continuing review application must be submitted to the IRB in a timely fashion and before expiry of this approval
5. Failure to submit a continuing review application will result in termination of the study
6. Notify the IRB committee once the study is finished

Sincerely,

Date of Approval: The 20th May 2020

Expiration date: The 20th May 2021

Professor GAHUTU Jean Bosco
Chairperson Institutional Review Board,
University of Rwanda College of Medicine and Health Sciences

Cc:

- Principal College of Medicine and Health Sciences, UR
- University Director of Research and Postgraduate Studies, UR

CENTRE HOSPITALIER UNIVERSITAIRE
UNIVERSITY TEACHING HOSPITAL

Ethics Committee / Comité d'éthique

August 16th, 2019 Ref: EC/CHUK/[illegible]/2019

Review Approval Notice

Dear Thomas HABANABANZE

Your research project: ***"Validation of the histological profile of hydatidiform moles using p57[KIP2] immunophenotyping and conventional karyotyping at the university Teaching Hospital of Kigali (CHUK)"***

During the meeting of the Ethics Committee of University Teaching Hospital of Kigali (CHUK) that was held on 5th August, 2019 to evaluate your request for application of ethics approval for the above mentioned research project we are pleased to inform you that the Ethics Committee CHUK has approved your research project.

You are required to present the results of your study to CHUK Ethics Committee before publication.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

ETHICS COMMITTEE
CHUK

Dr.RUSANGIZA KAMANZI Emmanuel
The Chairperson, Ethics Committee,
University Teaching Hospital of Kigali

<<University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (SOPs) which are approved on an annual basis and in compliance with GCP and Ethics guidelines and regulations>>

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CENTRE HOSPITALIER UNIVERSITAIRE

UNIVERSITY TEACHING HOSPITAL

CENTRE HOSPITALIER UNIVERSITAIRE DE BUTARE (CHUB)
OFFICE OF DIRECTOR GENERAL

Huye, ...23/03/2020

N° Ref: CHUB/DG/SA/07/1205/2020

Dr. Thomas HABANABAKIZE
Resident in Anatomical Pathology, Y3
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Email: drtom2020@gmail.com

Dear Habanabakize,

Re: Your request for data collection

Reference made to your letter requesting for permission to collect the data within University Teaching Hospital of Butare for your research project entitled ***"Profile of hydatidiform mole using histology, p57kip2 immunophenotyping and conventional karyotyping at University Teaching Hospitals of Kigali and Butare(CHUK, CHUB)"***, based to the approvals **No: 081/CMHS/IRB/2020** from Institution Review Board of University of Rwanda and **No: RC/UTHB/008/2020** from our Research-Ethics Committee, we are pleased to inform you that you are accepted to collect data within University Teaching Hospital of Butare. Please note that your final document will be submitted in our research office.

Sincerely,

Dr. Augustin SENDEGEYA
Director General of CHUB

Cc:

- Ag. Head of Clinical Education and Research Division
- Ag. Director of Research
- Head of Laboratory Department
- Ag. Research officer

CHUB