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| RESEARCH ARTICLE

A Study on the Diagnostic Value of p57kip2 among Gestational Trophoblastic Diseases at a Philippine Hospital in 2020

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| ABSTRACT

Hydatidiform mole (H. mole), a gestational trophoblastic disease, is common in the Philippines. In general, the differentiation between complete hydatidiform mole and partial hydatidiform mole is based on morphologic evaluation via routine H&E. The problem lies on the lack of a standard criteria and overlapping features. The p57kip2 immunohistochemical stain is inexpensive, locally available, and could provide reliable results in differentiating between complete H. mole and partial H. mole. This study aims to determine the accuracy of the diagnosis of H. moles by routine H&E stain as compared to the diagnosis by p57kip2 IHC stain. There were a total of 31 cases of H. moles in 2020. It includes 20 cases of complete H. moles and 11 cases of partial H. moles. With the advent of the p57kip2 IHC stain, a total of 18 cases were diagnosed as complete H. mole and 13 were diagnosed as partial H. mole. Diagnostic statistics such as sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), accuracy, and kappa statistics were computed to validate the diagnostic value of H&E staining with p57kip2 IHC staining on H. moles. This study concludes that a significant increase in accuracy and reliability are attained when supplemented with p57kip2 IHC stain. In addition, criteria in analyzing p57kip2 are simple and reproducible without significant interobserver variability. It is therefore recommended to use p57kip2 IHC stain on vesicular tissues to deduce the true nature of their pathology.

| KEYWORDS

Gestational trophoblastic disease, complete hydatidiform mole, partial hydatidiform mole, p57kip2

| ARTICLE INFORMATION

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1. Introduction

H. moles are relatively common in the Philippines. At our institution, we have about 30 to 60 diagnosed cases per year. There are no guidelines for the management of H. moles, whether it be complete or partial. In general, the management of these cases includes suction curettage, post-evacuation serial determination of serum B-hCG, and chemotherapy in cases of the development of persistent trophoblastic disease (PTD) or invasive moles, or worse, choriocarcinoma (Cavaliere, 2009). That being said, the risk of developing a malignancy between the two H. moles are significantly different. Complete H. moles have a 15% risk of developing choriocarcinoma, while partial H. moles have 0.5 % (Duffy, 2015). In addition to the usual management, patients who are misdiagnosed with complete H. mole will be subjected to unnecessarily prolonged contraception (Cavaliere, 2009). On the other hand, misdiagnosis of partial H. mole could lead to underestimation of potential risks and sequelae, insufficient further evaluation, and follow-up (Duffy, 2015). The study will include all vesicular and placental tissues submitted to the Bulacan Medical Center-Department of Pathology and Laboratory Medicine in the year 2020. All specimens diagnosed with 'Hydatidiform Mole,' 'Complete Hydatidiform Mole,' and 'Partial Hydatidiform Mole' will be included for further testing with immunochemical staining. Other diagnoses for the vesicular and placental tissues will not be included.

2. Literature Review

Gestational Trophoblastic Diseases (GTDs) are generally classified as either molar lesions or non-molar lesions (Kurman, 2011). The molar lesions include hydatidiform moles (H. moles), which are either complete or partial H. moles. The terminologies hydatidiform and mole were coined by William Smelie in 1752, which he described as a "bunch of grapes" that are varisized (Candelier, 2016).

Each of the H. moles is characterized based on clinical, morphologic, histochemical and genotypic features (Triratanachat, 2016). H. moles are a group of non-neoplastic lesions arising from abnormally formed placenta featuring significant hydropic enlargement, trophoblastic proliferation, and atypia of the chorionic villi (Diwa, 2016). H. moles are the most common type of GTDs, and the incidence has increased in Southeast Asia by about 3.2 – 9.9 for every 1,000 gestations (Kalsoom, 2015). They are classified as either complete or partial hydatidiform moles based on clinical, morphologic, histochemical and genotypic features (Triratanachat, 2016). H. moles may have the propensity to give rise to choriocarcinoma; hence the diagnosis of its true nature is very important (Kalsoom, 2015).

H. moles are common in the Philippines. A study done in 2006 revealed that the national prevalence rate of H. moles decreased from 7 per thousand gestations in the 1980s to 2.7 per thousand gestations between the years 1985 to 1994. There was a slight increase from 1997 to 2001 with a rate of 3.5 per a thousand gestations. According to a study by Cagayan, M., the prevalence rate at a certain tertiary government hospital in Manila, known to be a tertiary referral center for GTDs in the country, is 13.96 per thousand gestations (Cagayan, 2016). At our institution, the rate is approximately 12.9 per thousand gestations between the years 2018 to 2020.

The exact pathogenesis of H. moles are not fully known; however, it is the well studied type of GTD. It is thought to arise from excessive paternal haploid chromosomes. Complete H. moles show a 2:0 paternal/maternal ratio, while partial moles show a 2:1 ratio. Other factors associated with the disease entity include poor socio-economic status, malnutrition, history of prior molar pregnancy, and recently, asbestos exposure (Kurman, 2011).

In the Philippines, the diagnosis of H. moles is done based on identifying histomorphologic features via routine histopathology. However, the problem of misdiagnosis lies in the lack of standard criteria being used, with overlapping features between diagnoses, and thus suffering from interobserver variability. In general, genotyping remains to be the gold standard in diagnosing H. moles (Gupta, 2012). Unfortunately, this is rarely done due to cost and availability. As an alternative, immunohistochemical (IHC) tests are gradually being done to diagnose and differentiate not just GTDs, but also other disease entities. In relation to H. moles, as compared to its gold standard, IHC tests are deemed less expensive and more available. Of the IHCs available for GTDs, p57kip2 is seen as a promising IHC test for differentiating between complete and partial H. moles. P57kip2 is a product of the paternally imprinted but maternally expressed gene of CDKN1C on chromosome 11. It is a cyclin-dependent kinase inhibitor, which is a tumor supressor gene (Kalsoom, 2015).

3. Methodology

Vesicular tissue specimens were identified from the general histopathology logbook of the Department of Pathology and Laboratory Medicine at Bulacan Medical Center, Philippines, from Jan. 01, 2020, to Dec. 31, 2020. The accession number and routine H&E histopathologic diagnosis of each case were noted. A total of thirty-one vesicular tissues were identified, twenty cases were diagnosed via H&E as complete H. mole, and eleven cases were diagnosed via H&E as partial H. mole. With the use of the accession numbers, the slides and tissue blocks were retrieved. The slides were once again read by a pathologist to confirm the presence of H. mole. The tissue blocks were then submitted for processing at another institution for p57kip2 IHC staining. All of the p57kip2 IHC slides were then read by an IHC trained pathologist. The routine H&E histopathologic diagnosis was then compared to the p57kip2 IHC profile by computing diagnostic statistics such as sensitivity (Sn), specificity (Sp), positive predictive value (PPV), and negative predictive value (NPV). Kappa statistic was also computed with the use of GraphPad Software.

4. Results/Findings

4.1 Histopathology (H&E) Review

There were a total of 31 vesicular tissue specimens submitted for histopathologic evaluation in 2020. The diagnosis with H&E stain was 20 complete H. moles and 11 partial H. moles (Table 1).

Type	No of patients	Percentage
Complete H. mole	20	65%
Partial H. mole	11	35%
Total	31	100%

Table 1. Types of H. mole based on H&E stain

4.2 P57kip2 IHC Expression

A result of positive immunoreactivity was given when there was nuclear staining. Negative immunoreactivity was given when there was only cytoplasmic granular staining. A threshold of 10% strong staining of the total cytotrophoblastic cells was deemed positive, while below it was deemed negative (Kalsoom, 2015).

With the advent of the p57kip2 IHC stain, a total of 18 cases were diagnosed as complete H. mole, and 13 were diagnosed as partial H. mole (Table 2). Furthermore, 2 cases that were previously diagnosed as complete H. mole were reclassified as partial H. moles due to the lack of nuclear staining of the cytotrophoblasts, as well as the villous stroma, but with strong focal staining of the maternal decidua, deemed as our internal control (Figures 1, 2, and 3). None of the cases was reclassified from partial to complete H. mole. The partial H. moles were diagnosed due to the strong nuclear staining of the cytotrophoblasts (Figures 5 & 6).

Type	No of patients	Percentage
Complete H. mole	18	58%
Partial H. mole	13	42%
Total	31	100%

Table 2. Types of H. mole based on p57kip2 IHC stain

4.3 Statistical Analysis

Diagnostic statistics such as sensitivity (Sn), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV), and accuracy were computed to validate the diagnostic value of H&E staining with p57kip2 IHC staining on H. moles.

To obtain the sensitivity, the following formula is used:

$$Sn = \frac{\text{True Positive}}{\text{True Positive + False Negative}} = \frac{11}{11 + 2} = 84\%$$

To obtain the specificity, the following formula is used:

$$Sp = \frac{\text{True Negative}}{\text{True Negative + False Positive}} = \frac{18}{18 + 0} = 100\%$$

To obtain the positive predictive value, the following formula is used:

$$PPV = \frac{\text{True Positive}}{\text{True Positive + False Positive}} = \frac{11}{11 + 0} = 100\%$$

To obtain the negative predictive value, the following formula is used:

$$NPV = \frac{\text{True Negative}}{\text{True Negative + False Negative}} = \frac{18}{18 + 2} = 90\%$$

To obtain the accuracy, the following formula is used:

$$\text{Accuracy} = \frac{\text{True Positive + True Negative}}{\text{True Positive + True Negative + False Positive + False Negative}} = \frac{11 + 18}{31} = 93.55\%$$

Furthermore, with the use of GraphPad Software, Kappa statistic was done. This type of statistic is used to determine the agreement between repeat measurements having the same set of results, particularly when comparing results obtained differently. Kappa statistic measures agreement beyond chance and is also called an interrater reliability measurement (Williams, 1999).

Following the contingency table (Table 3), the number of observed agreements was 29.0 (93.55% of the observations). The number of agreements expected by chance was 16.2 (52.34% of the observations). Kappa was computed at 0.865, standard error of kappa at 0.092 95% confidence interval from 0.685 to 1.000.

The interpretations usually vary; however, poor reliability is often defined as a kappa of < 0.4, fair reliability as 0.4 - 0.6, good reliability as > 0.6 to 0.8, and very good as > 0.8 (10). In this study, the strength of the agreement is considered to be very good.

		IHC: p57kip2		
		Partial (IHC Positive)	Complete (IHC Negative)	Total
H&E	Partial	11	0	11
	Complete	2	18	20
	Total	13	18	31

Table 3. A contingency table showing the comparison between H&E stain results and p57kip2 IHC stain results.

Hydatidiform moles are the result of excessive paternal chromosomes in the product of conception. Thus, they are referred to as androgenetic. The only difference between complete and partial moles is that there is the participation of maternal chromosomes in the genetic make-up of the conceptus in partial moles, whereas, in complete moles, all chromosomes are derived paternally. It is this androgeneticity that confers their gross features, e.g. the markedly swollen villi. On the contrary, in parthenogenecity, all genomes are derived maternally and associated with opposite features. Both paternal and maternal genomes are necessary for the continuous development of an embryo; thus, fetal development is very rare in a complete mole.

Clinically, both the complete hydatidiform mole and partial have similar features, although complete moles tend to present with severe symptoms such as a large uterus disproportionate to the age of gestation, higher levels of beta-hCG, more severe vaginal bleeding, more prone to develop hyperthyroidism and eclampsia. Likewise, in gross and microscopic features, complete moles show greater histologic abnormality than partial mole, such as markedly swollen villi with central cisterns, absence of fetal tissues, and trophoblastic atypia. However, none of these features is exclusive, and there are overlaps even in the same specimen.

In this study, the need for standardized criteria for diagnosis was emphasized. This is due to the fact that all of the histologic features used to categorise hydatidiform mole as complete or partial are not objective and suffer from interobserver variability.

Differentiation between complete and partial mole has a significant clinical impact as it pertains to the development of persistent GTD and choriocarcinoma. P57 (kip2) is a protein product of the gene CDKN1C on chromosome 11 that is paternally imprinted but maternally expressed. This phenomenon is due to the higher activity of the gene product derived maternally(genetic imprinting). The p57 is a candidate tumor suppressor gene and regulator of cell growth, especially during fetal development, in which it inhibits the fetus from excessive growth. Thus, the absence of p57 expression as in complete moles is implicated in the increased incidence of neoplasm such as choriocarcinoma as compared to the lower incidence in partial moles and practically none in hydropic abortus.

With the concept of the karyotypic abnormalities of partial and complete moles, the application p57 is apparent. In this study, the interpretation of the p57 immunostains requires the assessment of the presence or absence of nuclear expression in villous stromal cells, cytotrophoblasts, intermediate trophoblasts and maternal deciduas. The p57 immunostain is interpreted as "positive" when the extent of staining is extensive or diffuse in the cytotrophoblasts and villous stromal cells. In addition to the typical diffusely positive result, 2 variants of positive staining can be encountered occasionally, equivocal and discordant. The p57 immunostain is interpreted as "equivocal" when nuclear expression in both villous stromal cells and cytotrophoblast is in the focally positive range (≥ 10% but <50% of the villi in the stained section). The p57 immunostain is interpreted as "discordant" when there is any combination/admixture of negative and positive results for villous stromal cells and cytotrophoblast within individual villi, including positive staining in cytotrophoblast and negative staining in villous stromal cells or vice versa.

As for the p57 immunohistochemical study, it was considered negative and satisfactory once the maternal decidua or intermediate trophoblastic cells' nuclei light up with p57. This then serves as the internal positive control in all the cases, including complete hydatidiform moles, while the villous stromal cells and cytotrophoblast are either entirely negative or demonstrated only limited expression. The negative result was then considered to be a complete hydatidiform mole. (Xing, 2022)

In this study, there were a total of 42 cases of hydatidiform mole. With H&E alone, 17 cases are diagnosed as complete moles and 25 cases as partial moles. With p57 immunostain, 5 cases of "partial mole" show no nuclear expression on their cytotrophoblasts and villous stroma; rather, expression was noted in maternal deciduas and peri-villous trophoblastic hyperplasias. Two (2) cases of "complete mole" show the nuclear expression on villi and cytotrohoblasts. No equivocal or discordant results were observed.

Several statistics are calculated to show the validity of the results of the H&E. Sensitivity summarizes how well the test detects disease. In this study, only 72% of the total cases diagnosed as partial moles were really partial moles, as confirmed by p57. Specificity measures how well the test identifies those who do not have the disease. In this study, only 88% of the total cases diagnosed as complete moles were really complete moles, as confirmed by p57.

Sensitivity and specificity are inherent properties of a test and are useful in describing its expected performance. But they can only be measured if the actual disease status of individuals is known. Naturally, when we apply the test in normal clinical practice, we

do not know who has the disease; we are using the test to help find out. Therefore, we are more interested in what a negative or positive test result means for the patient. For this purpose, we use predictive values. The positive predictive value (PPV) shows what fraction of patients who receive a positive test result actually have the disease. In this study, there is a 90% probability that the positive p57 result will have a partial mole. The negative predictive value shows how many people who receive a negative score really do not have the condition. In this study, there is a 68% probability that the negative p57 result will have a complete mole.

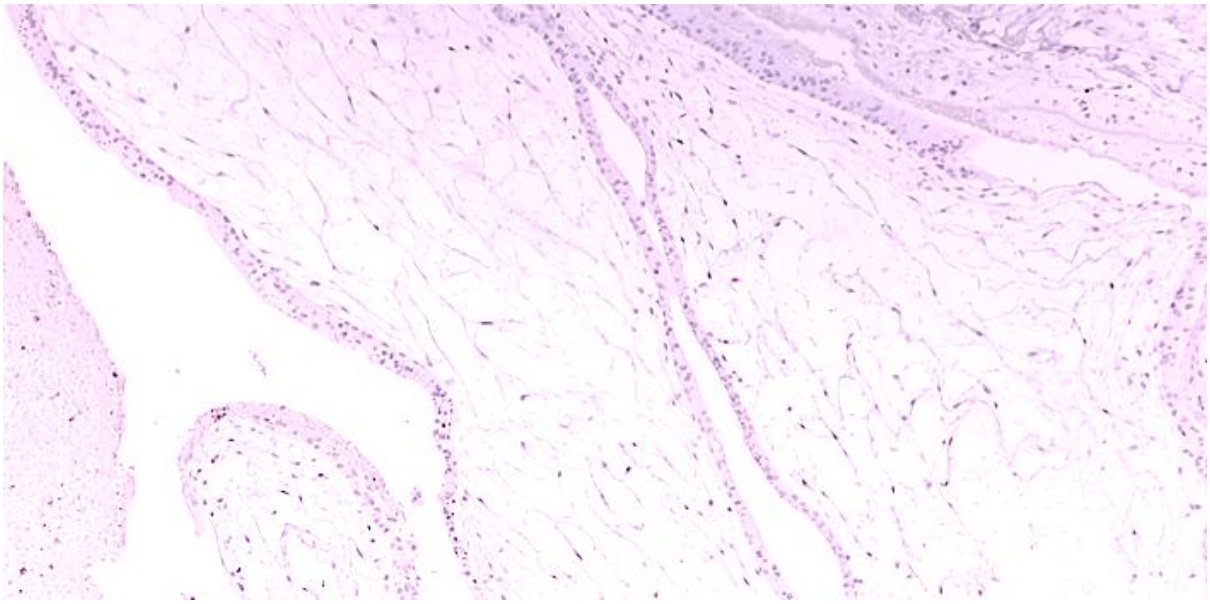


Figure 1. Complete H. mole. This photomicrograph shows negative p57kip2 expression on the cytotrophoblasts and villi. (LPO 10x)

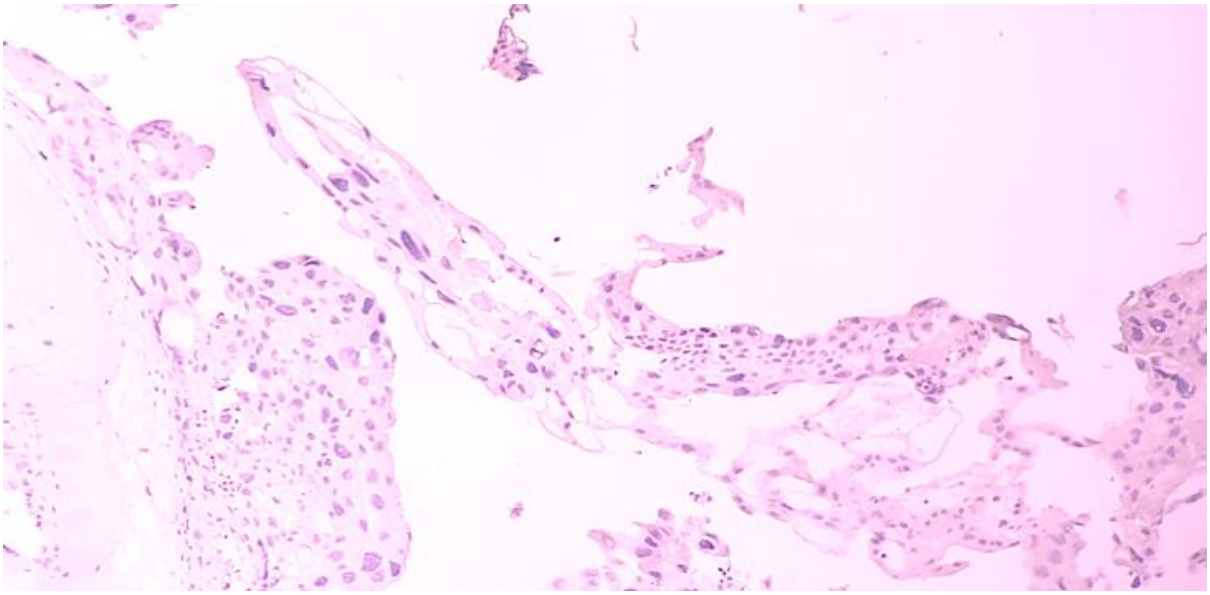


Figure 2. Complete H. mole. This photomicrograph shows negative p57kip2 expression on the villi, cytotrophoblasts and intermediate trophoblasts. (LPO 10x)

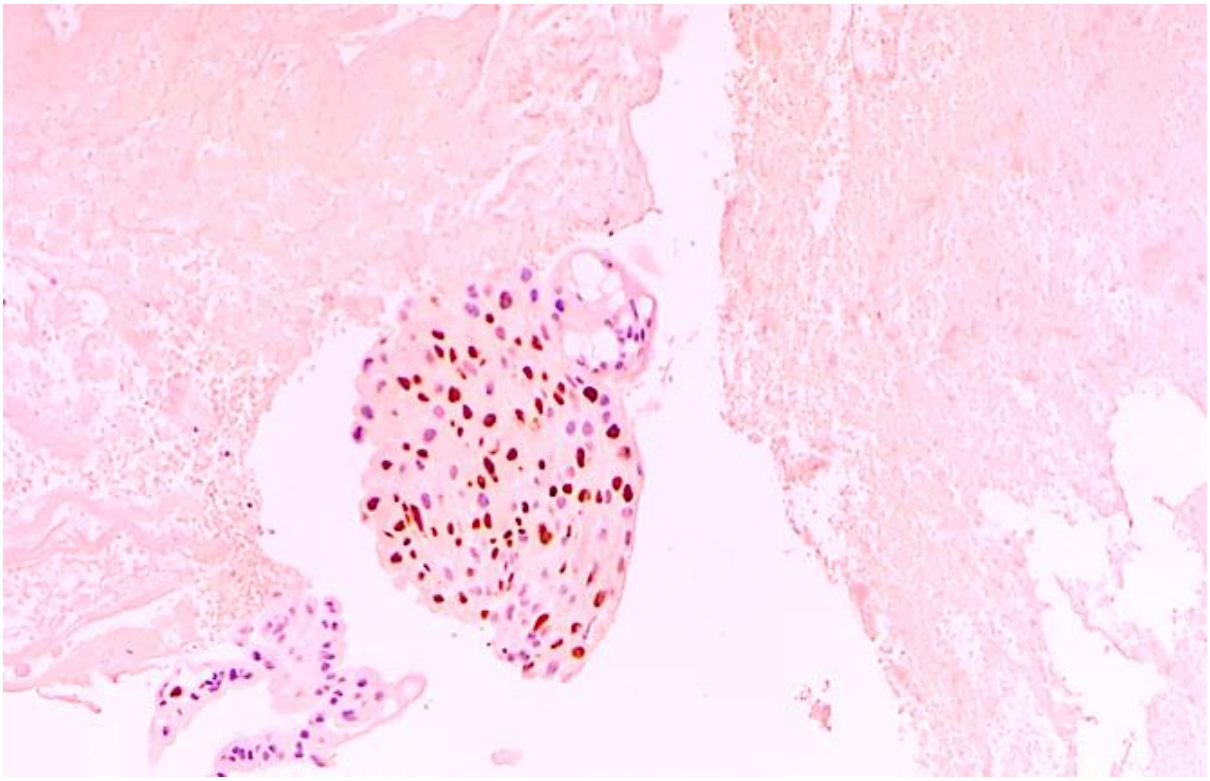


Figure 3. Complete H. mole. This photomicrograph shows p57kip2 nuclear expression on the maternal decidual cells. (LPO 10x)

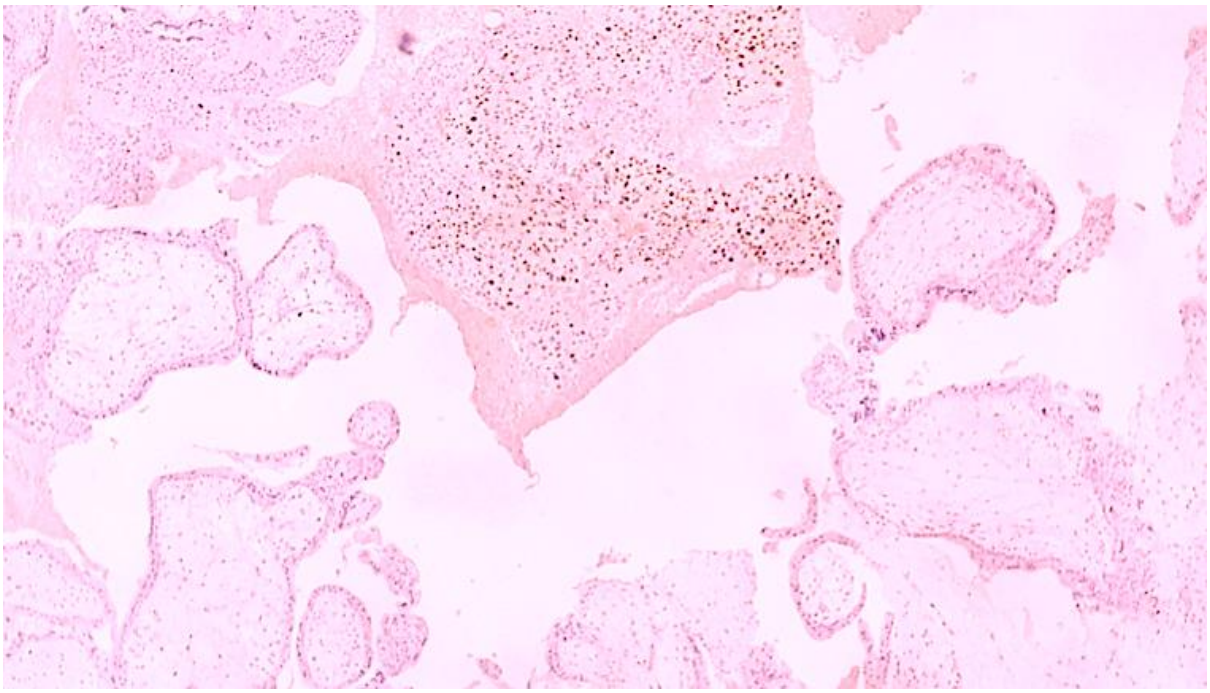


Figure 4. Complete H. mole. This photomicrograph shows negative p57kip2 nuclear expression cytotrophoblasts and villi. Trophoblastic proliferation is noted. (LPO 10x)

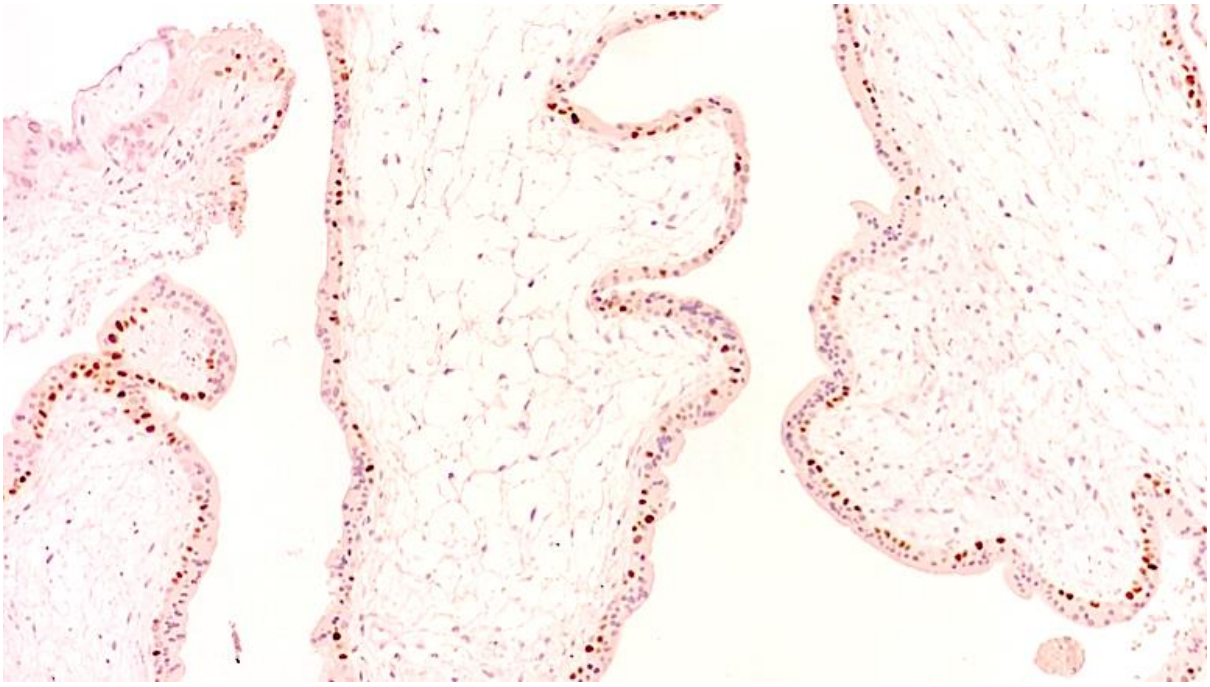


Figure 5. Partial H. mole. This photomicrograph shows strong p57kip2 nuclear expression of the cytotrophoblasts. (LPO 10x)

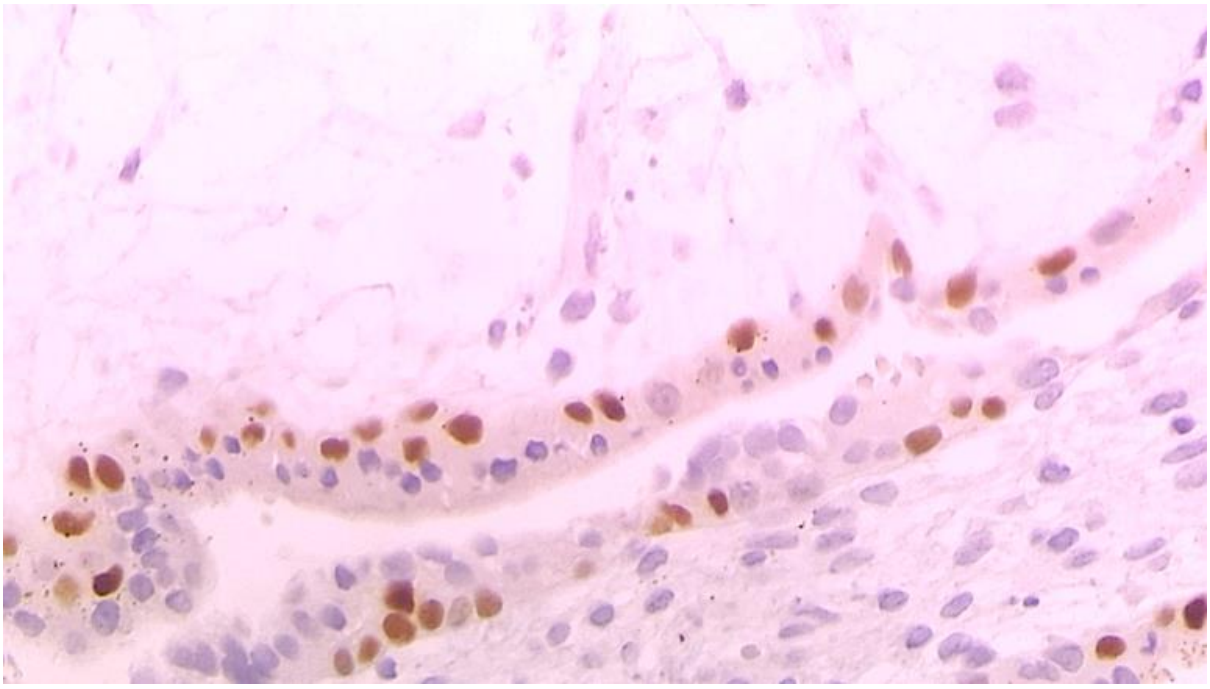
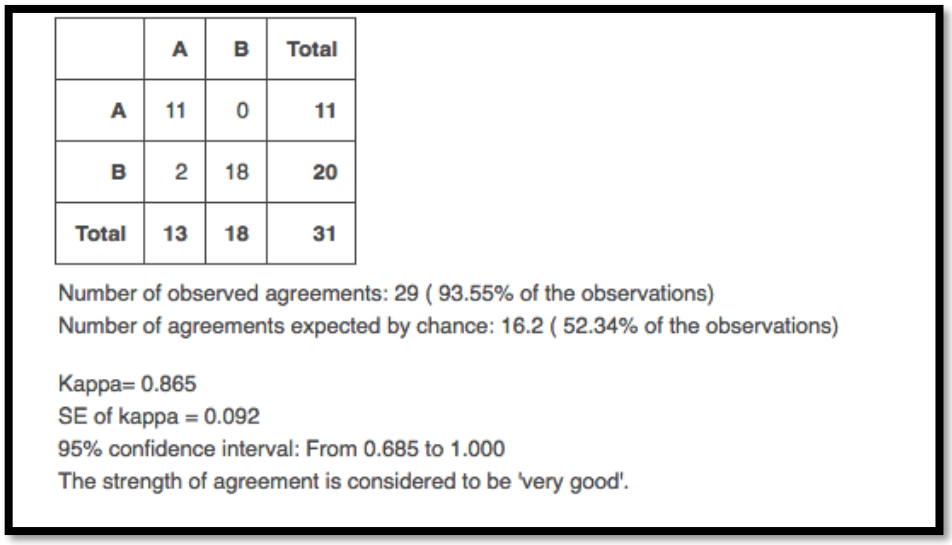


Figure 6. Partial H. mole. This photomicrograph shows strong p57kip2 nuclear expression of the cytotrophoblasts. (HPO 40x)



	A	B	Total
A	11	0	11
B	2	18	20
Total	13	18	31

Number of observed agreements: 29 (93.55% of the observations)
Number of agreements expected by chance: 16.2 (52.34% of the observations)

Kappa= 0.865
SE of kappa = 0.092
95% confidence interval: From 0.685 to 1.000
The strength of agreement is considered to be 'very good'.

Figure 7. Using GraphPad Software for computing the Kappa statistic. The strength of agreement is considered to be 'very good.'

5. Conclusion

The guidelines for the differentiation into partial and complete H. mole via morphological evaluation are not standardized. This study, therefore, aims to determine the validity of the diagnosis of H. moles by routine H&E stain as compared to the diagnosis by p57kip2 immunohistochemical stain. H&E stain is still the most readily available and cost-effective tool in diagnosing histopathologic lesions. However, based on this study proves that the adjunct use of p57kip2 immunohistochemical stain provides more reliable, objective, and reproducible results in differentiating between complete and partial H. moles. Again, it is important to determine the true pathology of these vesicular tissues being sent to our histopathology laboratory because the risk of developing a malignancy between the two H. moles is significantly different. Furthermore, patients who are misdiagnosed may be subjected to unnecessary management modalities. The analysis of p57kip2 expression, together with the correlation with complete history and other ancillary procedures, can differentiate more reliably and accurately between complete H. moles and partial H. moles. Due to the limited samples from a single institution, it is recommended that more samples from different hospitals be subjected to the p57kip2 immunohistochemical stain.

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Ethics: This study was approved by the institutional ethical review board of East Avenue Medical Center, Philippines, with IERB Protocol No.: EAMC IERB 2018 – 57.
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