ORIGINAL RESEARCH ARTICLE

Role of immunoexpression of cyclin D1, D3, retinoblastoma (Rb) mutant and clinical risk factors on complete moles as risk factors of persistent moles

Yudi M. Hidayat1*, Sofie R. Krisnadi1, Supriadi Gandamihardja1, Mieke H. Satari2, Bethy S. Hernowo3, Leri Septiani1, Ahmad Faried4

1Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Padjadjaran (FK UNPAD)–Dr. Hasan Sadikin Hospital (RSHS), Bandung, Indonesia
2Faculty of Dentistry, Universitas Padjadjaran, Bandung, Indonesia
3Department of Pathology Anatomy, FK UNPAD–RSHS, Bandung, Indonesia
4Oncology Working Group, FK UNPAD–RSHS, Bandung, Indonesia

Abstract: Introduction: Changes in complete hydatidiform mole (CHM) that become persistent are difficult to handle because the malignant pathogenesis of CHM is still unclear. The growth of abnormal cells in CHM is thought to be caused by cell cycle abnormalities. Some components that play a role in this phase include cyclin D and retinoblastoma (Rb). The aim of our study was to determine the role of clinical risk factors, as well as cyclin D1, cyclin D3 and Rb-protein, in the occurrence of persistent moles. Materials and Method: This study involves 68 CHM cases at Dr. Hasan Sadikin Hospital from 2007–2011. The protein expression of cyclin D1, cyclin D3, and Rb were determined by immunohistochemistry. The results were analyzed by comparing the two groups of CHM that became persistent to those that returned to normal, as determined by a Mochizuki regression curve assessment. Results: 20 cases (29%) of CHM became persistent and that 48 cases (71%) returned to normal. Significant clinical variables were age (p <0.05), histopathology (p <0.00) and βhCG (p <0.05). The immunoexpression of cyclin D1 (1.42), cyclin D3 (1.41) and mutant Rb (1.77) in the CHM cases that became persistent was higher than the CHM cases that returned to normal. The statistical analysis showed a significant difference in the immunoexpression of cyclin D1 (p ≤0.05) and Rb (p ≤0.05), whereas cyclin D3 immunoexpression were not significant (p >0.05). Conclusion: There is a strong relationship between clinical risk factors of age, excessive proliferation histopathology, serum βhCG levels ≥100,000 mU/mL, cyclin D1 and Rb mutations with the incidence of persistent moles after the evacuation of the CHM. We proposed a model to predict the risks of persistent moles with a cut-off point of 2.384, which can be used as a reference for patients with CHM.

Keywords: complete hydatidiform mole (CHM); persistent mole (PM); cyclin D1; cyclin D3; retinoblastoma (Rb); clinical risk factor

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*Correspondence to: Yudi M. Hidayat, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Padjadjaran–Dr. Hasan Sadikin General Hospital, Indonesia, yudiehma@yahoo.co.id.

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Trophoblasts are the main cells of the placenta. Gestational trophoblastic disease (GTD) is a disease that is derived from these cells. Complete hydatidiform mole (CHM) is a form of pathological trophoblastic cell disease that may potentially transition to malignancy. The incidence of benign or malignant trophoblastic disease in Indonesia and other developing countries is still high compared to developed countries[1-4]. The World Health Organization (WHO) reported that in Western countries, the incidence of hydatidiform mole...
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(HM) was approximately 1:1,450 to 1:2,000 pregnancies and the incidence of choriocarcinoma was 1:14,000 to 1:40,000[3]. However, in Indonesia, the incidence of HM was 1:51 to 1:141 pregnancies, and the incidence of the gestational trophoblastic tumor (GTT) was 1:822 pregnancies[3,5].

GTTs should be noted, especially when persistent moles are present after the evacuation of CHM, which has an incidence between 20% and 30%. This malignancy develops very progressively, with a mortality of 31%–51%[2,6,7]. The clinical risk factors that play a known role in post-mole malignancy include age ≥35 years, parity ≥4, uterine size ≥20 weeks, the presence of a lutein cyst, excessive histopathologic features of trophoblastic proliferation, and pre-evacuation serum βhCG level above 100,000 mU/mL; however, the detection of malignancy using the known clinical variables has not resulted in optimal outcomes[6,7]. Martaadiseoebatra[5] reported that the occurrence of choriocarcinoma after HM at age ≥35 years was higher (23.1%) than that of <35 years (17.9%). Similarly, Jayamasa et al.[9] reported that HM patients aged ≥35 years have a malignancy risk 2.1–3.8 fold higher than those aged <35 years[2,3,5,8].

One malignant form of initial GTT is persistent mole (PM) or persistent trophoblastic disease (PTD), according to The Federation International of Gynecology and Obstetrics (FIGO). It was reported that most women with GTT were 25–29 years old, and 82.7% of women with GTT were less than 40 years old[9]. The study by Perbawati et al.[10] in The Dr. Hasan Sadikin Hospital (RSHS) Bandung in 2010 reported that GTT patients in 2007 to 2009 were mostly of younger ages (25–29 years), with low parity, and most of the cases were preceded by CHM. In Indonesia, the efforts to reduce the incidence of GTT and to detect early pre-evacuation HMs have not been optimal, as indicated by the high incidence of post-HM malignancy, in both high risk and low risk groups. In RSHS, between 2007 and 2011, Sismawan and Hidayat[11] reported the incidence of GTT to be 38.2% in low risk groups and 61.8% in high risk groups. This indicates that the efforts to prevent malignancy based on past clinical risk factors have not been optimal and need improvement.

It has been shown that cyclin D1 and cyclin D2 play important roles in cellular proliferation, while cyclin D3 is involved in cellular differentiation. The impaired expression of cyclin D1, D2 and D3 was found in malignancies such as breast cancer, pancreatic ductal carcinoma, squamous cell carcinoma of head and neck and esophageal carcinoma. Currently, it is known that the tumor suppressor protein retinoblastoma (Rb) controls transcripational expression through an interaction with the protein E2F, which leads to the cooperation of Rb and E2F to inhibit the transcription of the mRNA of proteins that are required for DNA synthesis[12-20]. The above-mentioned molecular process is assumed to be the cause of malignant changes after HM and required further investigation.

Materials and methods

The study subjects were all patients with CHM from 2007 to 2011 and were hospitalized in RSHS Bandung. This study used a retrospective cohort design to investigate the association of demographic, clinical, laboratory and molecular risks of patients with CHM and malignancy occurrence (PM or GTT) based on βhCG monitoring after evacuation. Clinical risk factor variables, histopathology, laboratory results and gene expression profiles were obtained from medical records. These criteria were assumed to play a role in the incidence of persistent mole by applying the concepts of molecular biology that could be observed in the immunexpression of cyclin D1, cyclin D3 and Rb in the placenta of CHM. The patients with CHM were post-evacuatedly monitored for at least 6 months (for recovered patients) or up to 1 year in order to ascertain the development of PM or malignancy by the Mochizuki’s regression curve parameter of βhCG.

Immunohistochemistry (IHC). Primary tumor samples were fixed in 10% (v/v) formalin, embedded in paraffin and 4 μm sections were processed with hematoxylin eosin (H&E). The tissue was evaluated by histological examination under a light microscope. Tissue sections were treated with monoclonal anti-cyclin D1, anti-cyclin D3 and anti-Rb mutant antibodies for the immunohistochemical analysis. The sections were examined by light microscopy to establish the presence or absence of immunostaining and its distribution.

Statistics. The study results were statistically analyzed with an unpaired chi-square test or t-test to compare two groups of post-evacuation moles (i.e., PM or returned to normal based on the Mochizuki’s regression curve assessment). The statistical analysis was performed using SPSS, version 12.0 (SAS Institute, Cary, NC). p <0.05 was considered to be significant.

Results

The study was conducted to investigate the role of immunexpression of cyclin D1, cyclin D3 and mutant Rb, as well as the clinical risk factors for the occurrence of persistent mole or malignancy in patients after hyda-
tidiform mole evacuation. 146 study subjects were recruited and consisted of patients with diagnosed CHM that were curetted at the Department of Obstetrics and Gynecology, RSHS Bandung. Of the 68 CHM cases, 20 cases (29%) developed into PM, while 48 cases (71%) returned to normal, which became the control group (Table 1).

| Table 1 Number of CHM cases that met the inclusion criteria |
|-----------------|------|------|
| Complete hydatidiform mole | N   | %    |
| Become persistent mole    | 20  | 29   |
| To be normal (regression) | 48  | 71   |
| Total                       | 68  | 100  |

There were higher histoscore values in the CHM cases with persistent moles than in the cases that became normal based on the classification of cyclin D1, cyclin D3 and mutant Rb immunoeexpression: cyclin D1 (5.4 vs. 3.8), cyclin D3 (10.9 vs. 7.7), mutant Rb (10.3 vs. 5.8). immunoeexpression of cyclin D1, cyclin D3 and mutant Rb in CHM that became PM were higher compared to those that became normal (regression) (Table 2).

The ratio value was 1.42 for cyclin D1, 1.41 for cyclin D3, and 1.77 for mutant Rb. Statistical tests indicated a significant difference in the proportion of cyclin D1 ($p = 0.021$) and Rb ($p = 0.024$), whereas in cyclin D3, there was no significant difference ($p > 0.05$). The associations between clinical variables and the occurrence of PM using a chi-square test are displayed in Table 3. A bivariate analysis was used for variables of parity, lutein cyst and uterine size, which showed no statistically significant difference ($p > 0.05$); whereas age ($p = 0.04$), excessive histopathologic features of trophoblastic proliferation ($p = 0.00$), and pre-evacuation serum βhCG level ($p = 0.01$) were significant, implying a high significance at $p < 0.05$ (Table 4). A cross-tabulated calculation between clinical variables and immunoeexpression

| Table 2 Immunoeexpression Cyclin D1, D3, and mutant Rb in CHM-to-be-PM group and in CHM-to-be-normal group (Regression) |
|-----------------|------|------|------|------|------|------|
| Immunoeexpression (Histoscore) | CHM-to-be-PM | CHM Regression |
|                  | Total score | Mean | SD  | Total score | Mean | SD  |
| Cyclin D1        | 108      | 5.4  | 4.97 | 181      | 3.8  | 3.74 |
| Cyclin D3        | 217      | 10.9 | 1.89 | 371      | 7.7  | 2.96 |
| Rb mutant        | 206      | 10.3 | 3.31 | 276      | 5.8  | 4.84 |

Notes: Immunoeexpression IHC are classified according to histoscore calculation.

| Table 3 The ratio difference of immunoeexpression in CHM-to-be-PM group and in CHM-to-be-normal group (regression) |
|-----------------|------|------|------|------|
| Immunoeexpression (Histoscore) | N | Persistent mole (mean) | Regression (mean) | Ratio |
| Cyclin D1        | 68  | 5.4  | 3.8  | 1.42  | 0.021* |
| Cyclin D3        | 68  | 10.9 | 7.7  | 1.41  | 0.683  |
| Retinoblastoma (Rb) | 68  | 10.3 | 5.8  | 1.77  | 0.024* |

Notes: Immunoeexpression IHC are classified according to histoscore calculation for immunoeexpression data, $p$ is calculated based on Mann-Whitney and Wilcoxon test. The significance value based on $p$ value $<0.05$. *(asterisk) implies significance statistically.

| Table 4 The clinical characteristics of the subjects that become persistent mole |
|-----------------|------|------|------|------|
| Clinical characteristics | N | Regression | Persistent mole | $p$ |
| Age (year)       |    | % | % |    |
| <35              | 40  | 30 | 62.6 | 10 | 50 | 0.04* |
| $\geq$35         | 28  | 18 | 37.4 | 10 | 50 |    |
| Parity           |    | % | % |    |
| <4               | 15  | 8  | 16.7 | 7  | 35 | 0.14 |
| $\geq$4          | 53  | 40 | 83.3 | 13 | 65 |    |
| Lutein Cyst      |    | % | % |    |
| (−)              | 62  | 45 | 93.7 | 17 | 85 | 0.24 |
| (+)              | 6   | 3  | 6.3  | 3  | 15 |    |
| Histopathological figure of trophoblastic proliferation | Not excessive | 48 | 44 | 91.7 | 4 | 20 | 0.00* |
| Excessive        | 20  | 4  | 8.3  | 16 | 80 |    |
| Uterine Size     |    | % | % |    |
| <20 weeks        | 37  | 27 | 56.3 | 10 | 50 | 0.42 |
| $\geq$20 weeks   | 31  | 21 | 43.7 | 10 | 50 |    |
| Serum βhCG serum |    | % | % |    |
| <100,000 mU      | 26  | 23 | 47.9 | 3  | 15 | 0.01* |
| $\geq$100,000 mU | 42  | 25 | 52.1 | 17 | 85 |    |

Notes: For clinical and demographic data, $p$ was calculated based on chi-square test. The significance value based on $p$ <0.05. *(asterisk) implies significance statistically.
provided significance values for the occurrence of PM with \( p < 0.05 \), which also indicates significant association between the clinical variable of \( \beta hCG \) and histopathological features, such as the immunoeexpression of cyclin D1 and mutant Rb at \( p < 0.05 \) (Table 5).

A multivariate analytic calculation using logistic regression indicated three clinical variable outcomes (i.e., age, histopathologic figure and serum \( \beta hCG \) level). The expression of two proteins (i.e., cyclin D1 and mutant Rb; Figure 1) were significantly associated with the occurrence of persistent moles (\( p < 0.05 \)), whereas parity, uterine size, lutein cyst and immunoeexpression of cyclin D3 showed no significant association (\( p > 0.05 \)) (Table 6).

From the above-mentioned multivariate analysis, \( z \) values were calculated and determined to be the basic to calculate the role of each variable, weighted by risk factors, that influences the occurrence of PM. The weighted values of each variable in Table 7 include the lowest \( z \) value, \( \beta hCG \) level \( z = 0.50 \) was given a weight of 1 (0.5 \( \times 2 \times 10 \)), while the other risk factors were calculated by dividing the \( z \) value of each variable by the \( z \) value of the serum \( \beta hCG \) level. For example, consider a weighted age value with \( z = 2.02 \) (2.02/0.5 \( \times 10 \)), resulting in score of 40; a weighted \( z \) parity value = \(-1.46/0.5 = -2.9 \times 10 = -29 \) and so on, providing the results listed in Table 7.

### Table 5

| Clinical variables                  | Immunoeexpression | \( p \)  |
|------------------------------------|-------------------|--------|
| Pre-evacuation \( \beta hCG \) \( \geq 100,000 \) mU/mL | Cyclin D1         | 0.026* |
| Pre-evacuation \( \beta hCG \) \( \geq 100,000 \) mU/mL | Mutant Rb         | 0.006* |
| Histopathological figure of excessive trophoblastic proliferation | Cyclin D1         | 0.048* |
| Histopathological figure of excessive trophoblastic proliferation | Mutant Rb         | 0.039* |

Notes: The cross-tabulated calculation between immunoeexpression IHK are classified according to histoscore and clinical variables, \( p \) is calculated based on chi-square test. The significance value based on \( p <0.05 \). *(asterisk) implies significance statistically.

### Table 6

| Variable                      | Coefficient | \( Z \) | \( p >|z| \) | Odds ratio | IK |
|-------------------------------|-------------|--------|--------------|------------|----|
| Age                           | 0.19        | 2.02   | 0.04*        | 1.21       | 0.00 |
| Parity                        | -0.78       | -1.46  | 0.14         | 0.45       | -1.85 |
| Uterine size                  | 0.23        | 1.59   | 0.11         | 1.26       | -0.05 |
| Histopathological figure      | 4.41        | 3.23   | 0.00*        | 82.31      | 1.73 |
| \( \beta hCG \) level         | 0.65        | 0.50   | 0.01*        | 1.92       | -1.88 |
| D1 histoscore                 | 0.01        | 2.34   | 0.02*        | 1.01       | 0.00 |
| Rb histoscore                 | 0.01        | 1.68   | 0.03*        | 1.01       | -0.00 |
| Constant                      | -16.87      | -2.82  | 0.00         | -28.60     | -28.60 |

Notes: For multivariate data, clinical variables and immunoeexpression, \( p \) is calculated based on chi-square test then conducted multivariate using logistic regression. The significance value based on \( p <0.05 \). *(asterisk) implies significance statistically.
The calculation of cutoff point, sensitivity and specificity

Table 8 The calculation of cutoff point, sensitivity and specificity

| Classification | Cut-off point | Sensitivity | Specificity | Post-correction | LR+ | LR− |
|----------------|--------------|-------------|-------------|-----------------|-----|-----|
|                | (≥2218)      | 85.00%      | 58.33%      | 66.18%          | 2.0400 | 0.2571 |
|                | (≥2309)      | 85.00%      | 60.42%      | 67.65%          | 2.1474 | 0.2483 |
|                | (≥2312)      | 85.00%      | 62.50%      | 69.12%          | 2.2667 | 0.2400 |
|                | (≥2324)      | 85.00%      | 64.58%      | 70.59%          | 2.4000 | 0.2323 |
|                | (≥2330)      | 85.00%      | 66.67%      | 72.06%          | 2.5500 | 0.2250 |
|                | (≥2338)      | 80.00%      | 66.67%      | 70.59%          | 2.4000 | 0.3000 |
|                | (≥2343)      | 80.00%      | 68.75%      | 72.06%          | 2.5500 | 0.2909 |
|                | (≥2344)      | 80.00%      | 70.83%      | 73.53%          | 2.7429 | 0.2824 |
|                | (≥2347)      | 75.00%      | 70.83%      | 72.06%          | 2.5714 | 0.3529 |
|                | (≥2361)      | 75.00%      | 72.92%      | 73.53%          | 2.7692 | 0.3429 |
|                | (≥2384)      | 75.00%      | 75.00%      | 75.00%          | 3.0000 | 0.3333 |
|                | (≥2385)      | 70.00%      | 75.00%      | 73.53%          | 2.8000 | 0.4000 |
|                | (≥2397)      | 70.00%      | 77.08%      | 75.00%          | 3.0545 | 0.3892 |
|                | (≥2421)      | 65.00%      | 77.08%      | 73.53%          | 2.8364 | 0.4541 |
|                | (≥2478)      | 65.00%      | 79.17%      | 75.00%          | 3.1200 | 0.4421 |
|                | (≥2523)      | 65.00%      | 81.25%      | 76.47%          | 3.4667 | 0.4308 |
|                | (≥2527)      | 65.00%      | 83.33%      | 77.94%          | 3.9000 | 0.4200 |
|                | (≥2547)      | 60.00%      | 83.33%      | 76.47%          | 3.6000 | 0.4800 |
|                | (≥2554)      | 60.00%      | 85.42%      | 77.94%          | 4.1143 | 0.4683 |
|                | (≥2646)      | 60.00%      | 87.50%      | 79.41%          | 4.8000 | 0.4571 |
|                | (≥2695)      | 60.00%      | 89.58%      | 80.88%          | 5.7600 | 0.4465 |

Discussion

The analytical results of the demographic, clinical variables and expression in this study are as follows:

Serum βhCG level ≥100,000 mU/mL. The proliferation of trophoblastic cells, mainly syncytiotrophoblast, will enhance the production of βhCG, leading to an elevated serum βhCG level. An elevated serum βhCG level ≥100,000 mU/mL is a risk factor variable that is significantly associated with the incidence of PM (p = 0.01). In the calculation of serum βhCG levels as one of the malignancy risk factors, z = 0.5 was the lowest z value, implying that this variable is the most important compared to other variables; thus, serum βhCG levels can be applied as a reference to calculate the score magnitude of other risk factor variables, i.e., if the z value is 1 (from 2 × 0.5), then other risk variables should be multiplied by 2.

Age at gestation ≥35 years. Age had a significant association with the occurrence of PM, with p = 0.04 and z = 2.02 in this study. Thus, it can be statistically concluded that there was a positive effect of age on PM with z + 2.02. This result suggests that CHMs at age ≥35 have a 4-fold higher risk of malignant development after mole evacuation compared to the risk factor of serum βhCG level ≥100,000 mU/mL. Age was also implicated as a risk factor for the incidence of CHM pregnancy.

Parity ≥4. Parity had no significant association with PM occurrence. Parity was a higher risk factor for CHM pregnancy (p > 0.05 and z = −1.46). In this study, it was statistically found that the protective effect of high parity (≥4) on PM occurrence was shown by the negative z value of 1.46. This suggests that parity ≥4 has a 2.9X (1.46/0.5) greater protective effect against the incidence of malignancy after mole evacuation compared to the serum βhCG level ≥100,000 mU/mL risk factor.

Uterine size ≥20 weeks. Uterine size was a risk factor for PM prediction, although it should be carefully applied because not all CHM patients were admitted with the correct uterine size, and the statistical analysis showed no significant association (p = 0.23; z = +1.59). Multivariate analysis found z = +1.59; therefore, the statistically positive effect of uterine size at ≥20 weeks on the incidence of post-HM evacuation was 3.2 (2 × 1.59) times greater than serum βhCG levels in CHM patients. Uterine size was the secondary symptom with occurrence of excessive trophoblastic cell proliferation in the uterine space; the more the trophoblastic cells formed, the larger the size of the uterus.

Lutein cyst. The variable of lutein cyst did not appear to be a risk factor variable for PM because there was no...
significant association with PM occurrence. Cysts within ovaries that form due to hCG stimulation which require over time are lutein cysts. In the case of CHMs that are promptly treated by evacuation at a hospital, hCG stimulation will immediately disappear, without the development of lutein cysts. The statistical analysis did not show a significant association \( p > 0.05 \).

**Histopathologic figure of excessive trophoblastic proliferation.** The histopathologic figure of excessive trophoblastic proliferation is a manifestation of clinical processes occurring at the molecular level due to an uncontrolled cell cycle that leads to uncontrolled cellular proliferation. In this study, there was a significant association between the histopathologic figure of excessive trophoblastic proliferation and PM occurrence \( (p = 0.00) \), \( z = 3.23 \). This suggested that the histopathologic figure of excessive trophoblastic proliferation has a statistically positive risk effect that is 6.5X greater than the hCG level of malignant occurrence after mole evacuation.

**Immuinoexpression of cyclin D1.** In this study, the immunoexpression of cyclin D1 had a significant association with PM \( (p = 0.02-0.03) \), and there was a difference in the ratio of immunoexpression levels. The histoscore level of cyclin D1 was higher in CHM patients who developed PM compared to CHM that returned to normal \( (5.40:3.77; 1.43X) \). The enhanced immunoexpression of cyclin D1 affects the cell cycle rate in CHM, causing phase progression of G1/S within the cell cycle, which leads to rapid uncontrolled cell cycle function. In the multivariate analysis, the \( z \) value for cyclin D1 was \( +2.34 \), suggesting that the immunoexpression of D1 in CHM had a 5X greater effect than that of the hCG level on PM incidence in patients after CHM evacuation.

**Immuinoexpression of cyclin D3.** In our study, immunoexpression of cyclin D3 had no statistical association with PM \( (p > 0.05) \), but there was a difference in the expression ratio of the cyclin D3 histoscore, which was higher in CHMs developing to PM compared to CHMs that returned to normal \( (10.85:9.93; 1.09X) \). The immunoexpression of cyclin D3 did not show significant differences, which might be due to the monitoring time period being too short (one year). It has been shown that cyclin D3 plays a role in the cellular differentiation and trophoblastic cellular differentiation in moles that will become choriocarcinoma, invasive moles and placental site trophoblastic tumors that require a longer period. The elevation of cyclin D3 expression together with cyclin D1 influences the cell cycle rate in CHM, causing phase progression of G1/S in the cell cycle and leading to rapid cycling and aberrant cellular differentiation. In our study, the immunoexpression of cyclin D3 was not considered to be a predictive factor in malignancy because there was no statistically significant association.

**Immuinoexpression mutant Rb.** From these study results, the immunoexpression of mutant Rb was significantly increased and it had a highly significant association with malignant incidence \( (p = 0.03) \). The immunoexpression of mutant Rb is very important to know due to its abnormality and role as a key cell cycle regulator, especially in cycle phase G1/S. In a multivariate analysis, the \( z \) value was \( +1.68 \), which implied that the immunoexpression of mutant Rb affected the incidence of persistent moles or malignancy after CHM evacuation 3X more than hCG. From all the variables that affected the process of PM after CHM evacuation in this study, a table model of PM risk prediction was developed with the following model:

From the model, a cut-off value of 2.384 can be derived; therefore, the interpretation of scoring of PM risk prediction is as follows:

- Total score <2.384: the patient has complete hydatidiform mole (CHM) with low risk.
- Total score ≥2.384: the patient has complete hydatidiform mole (CHM) with high risk.

**Conclusion**

Based on these study results, it can be concluded that the immunoexpression of cyclin D1 was stronger in CHM patients who developed to PM compared to CHM patients who returned to normal (regression). There was no significant association between the increased risk factor of immunoexpression of cyclin D3 in PM after CHM evacuation. Immunoexpression of mutant Rb was stronger in CHM patients who developed to PM compared to the CHM patients who returned to normal (regression). There was a strong association of clinical risk factors (age, histopathologic figure of excessive proliferation, serum \( \beta hCG \) level \( \geq 100,000 \) mU/mL, immunoexpression of cyclin D1 and immunoexpression of mutant Rb) with PM incidence. The clinical variables identified that affect PM incidence were age \( \geq 35 \) years with weight \( (40) \), \( \beta hCG \geq 100,000 \) mU/mL \( (10) \), the histopathological figure of excessive trophoblastic cell proliferation \( (65) \), cyclin D1 \((47)\) and mutant Rb \((34)\).

Certain women who become pregnant at an older age \( (\geq 35) \) years may develop pathological pregnancies, such as CHM pregnancy. The molecular processes of the increased immunoexpression of cyclin D1, which affects the cell cycle rate (especially phase G1/S), and the compromised tumor suppressor function (Rb mutation) are key factors in understanding the pathogenesis of persis-
tent mole. We developed an application model of risk prediction of persistent moles from clinical variables and immunoexpression patterns that influence persistent mole incidence with a cut-off point at 2.384. This model of risk prediction of persistent mole at the Bandung Trophoblastic Centre resulted in the stratification of high-risk and low-risk CHM patients.

**Conflict of interest**

The authors declared no potential conflict of interest with respect to the research, authorship, and/or publication of this article.

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