Within 24 hours of life the infant showed no sign of viscous obstruction nor perforation.
The fetus was found to have an enlarged scrotum containing a large fluid collection with densities of echogenicity, highly suggestive of calcifications.

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- Subchorionic Hematoma in Threatened Abortion as Risk Factors Occurrence of Spontaneous Abortion
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- Relation between C-Reactive Protein Level and Intrauterine Infection in Pregnant Women with Premature Rupture of Membrane (PROM)
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- Correlation between Content of Collagen I and Tenascin-C Sacrouterine Ligament in the Uterine Prolapse
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- The Correlation between p53 Expression and Number of Fibroblast at Uterosacral Ligament of Women with and without Uterine Prolapse
- Prevalence of Positive Visual Inspection Acetic Acid Application (VIA) and its Association with Cervical Cancer Risk Factors among Women Visiting Kramat Jati Community Health Center Over a Three-day Screening in December 2011
- The Comparison of Expression of Cyclin D and Retinoblastoma Mutant Protein in Hydatidiform Mole and in Normal Placenta
- Polymorphism Estrogen Receptor α. Gene of Epithelial Ovarian Carcinoma
- Clinicopathologic Factor, Endostatin Serum Level, and Vascular Endothelial Growth Factor-C (VEGF-C) Serum Level as Predictors of Lymph Nodes Metastasis in Early Stage Cervical Cancer Patients
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**Case Reports**

- Prenatal Diagnosis and Postnatal Management of Meconium Peritonitis

**Literature Review**

- Ovarian Reserve Tests: The Use in Daily Clinical Practice
The Comparison of Expression of Cyclin D and Retinoblastoma Mutant Protein in Hydatidiform Mole and in Normal Placenta

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Abstract

Objective: To know the expression of cyclin D1 and mutant retinoblastoma in hydatidiform mole and to know the pathogenesis of hydatidiform mole pregnancy.

Method: Research specimens were taken from hydatidiform mole trophoblastic tissue in Dr. Hasan Sadikin Hospital and networking. Specimen preparations were stained with Immunohistochemistry and examined under a light microscope without knowing the status of the patient. Significance of the result was tested through the Mann-Whitney test, Fisher exact, and McNemar square test.

Result: Significant differences in the expression of cyclin D1 between the hydatidiform mole study groups and normal placental tissue research groups (p<0.001). There were significant differences in the expression of mutant retinoblastoma between the hydatidiform mole study groups and normal placental tissue research groups (p<0.05). There were significant differences between the domination of cyclin D1 and mutant retinoblastoma in hydatidiform mole tissues.

Conclusion: The expression of retinoblastoma was found different from normal histopathologically and it was suspected as mutant retinoblastoma. Expression of cyclin D1 and mutant retinoblastoma in hydatidiform mole trophoblast tissue increased, with mutant retinoblastoma being more dominant.


Keywords: expression of cyclin D1, retinoblastoma mutant, and hydatidiform mole

INTRODUCTION

Gestational trophoblastic disease is a group of diseases related to chorialis villous trophoblast cells in particular, consist of complete hydatidiform mole and partial hydatidiform mole which are benign and invasive mole, choriocarcinoma, placental site trophoblastic tumors which are malignant.1-5

Incidence of diseases both benign and malignant trophoblast in Indonesia and several other developing countries is still high compared with developed countries. Based on research data from WHO, in developed countries there were hydatidiform mole incidence of between 1:1450 to 1:2000 pregnancies, whereas in Japan there were 2 cases per 1000 pregnancies in which 3 times higher than Europe and North America which was about 0.6 to 1 and, 1 per 1000 pregnancies. When compared with developed countries and other developing countries, it appears that the incidence of hydatidiform mole in Bandung was quite high, 1: 427.5

Several factors may affect the occurrence of mole are age, parity, previous molar pregnancy, twins pregnancy, ethnic, and genetic.2,6 Mc Connell et al examined the role of gene abnormalities during trophoblast cell proliferation resulting in a failure of pregnancy that produces a form of malignancy. An imbalance between the tumor supressor gene and growth factor in trophoblast cell cycle, makes excessive proliferation toward malignancy.7,10

The cause of the normal development of trophoblast cells to become abnormal until now is still studied, suspecting the role of genetic factors. This became the basic for researchers to find the cause of hydatidiform mole associated with a factor of genetic changes through an initial examination of immunohistochemical staining in hydatidiform mole trophoblast tissue that contained the expression of tumor suppressor genes and growth factor is excessive compared with normal placental tissue.
Tumor suppressor genes (TSG) is a protein that plays a role to control and stop the cell cycle. TSG which sorts hydatidiform moles contained in them is Retinoblastoma, p53, GAP (GTPase activating protein). Retinoblastoma (Rb), a protein that functions as a suppressor gene was first discovered in patients with retinoblastoma. Mutations of this gene can be found in patients with breast cancer, lung cancer, renal cancer, bone cancer. This gene in normal circumstances, binds to the transcription factor E2F, the complex is located on the G0 and G1 phase. Rb-E2F complexes split, regardless E2F-free. E2F transcription factor that escapes becomes active, and is instrumental in stimulating the cell cycle to enter the stage of S phase and E2F transcription factors are also required for DNA replication. The entry of the viral genes for example the SV40 virus infection, adenovirus and viral infection7,10,12, may also inactivated Rb.

If the function of TSG is missing then the cell cycle and cell proliferation are not controlled, and makes an abnormal condition such as hydatidiform mole. Studies on the expression of mutant retinoblastoma and p53 mutant showed an increase in hydatidiform mole, which means that the control mechanisms of trophoblast cells from placental trophoblast tissue can develop into a pregnancy failure, in this case a hydatidiform mole. But if not found expression of retinoblastoma mutant or mutant p53, then the risk of degenerating into the abnormality is low. Protein is very important to suppress the cell cycle, which is the primary control mechanism of cell proliferation.

Growth Factor is a protein that has a role to activate and stimulate the cell cycle and cell proliferation. One of the trophoblast tissue growth factor is a cyclin, a protein which levels fluctuate at each phase of the cell cycle. Cyclin forms a complex with cyclin-Dependent Protein Kinase (CDK) proteins which are active, and play a role in the process of phosphorylation of specific protein substrates (Rb).2,8,9 There are seventeen of cyclin proteins that have been identified, working on the phases of the cell cycle. Cyclin-dependent protein kinase (CDK) is an enzyme that plays a role in catalyzing the process of protein phosphorylation. Phosphorylation process is an important part in the activation or inactivation of a number of proteins that play a role in cell cycle. Each CDK forms a complex with cyclin to become active. CDK4 and cyclin D1, D2, and D3 catalyze the phosphorylation of Rb protein, thus E2F previously is not active because it binds to the Rb. E2F is released and becomes active. Some also binds to cyclin CDK2, CDK5 and CDK6. CDK2 complexes with cyclin E1 or E2, will stimulate the regulatory cells from G1 phase to S phase, CDK1 complexes with cyclin B1 or B2 would regulate the G2 phase to phase M.10,11

The central theme in this study is the difference in the expression of cyclin proteins (growth factors) and mutant retinoblastoma (a tumor suppressor gene) on trophoblastic tissue, as a cause of hydatidiform mole. Cyclin D and retinoblastoma mutants are factors that play a role in trophoblast tissue cell cycle. Excessive expression of both causes excessive proliferation of trophoblast tissue which resulted in a hydatidiform mole. Immunohistochemical examination of an initial examination was done to determine the expression of both proteins.

## METHODS

Analytic observational study aimed to determine whether there are differences in protein expression levels of cyclin D1 and Retinoblastoma mutants in hydatidiform mole trophoblastic tissue compared with normal placenta.

Research subjects were patients with hydatidiform mole at Dr. Hasan Sadikin hospital Bandung and networking during the study period. Sampling of this study was based on the order of arrival of patients including exclusion and inclusion criteria and the minimum sample size. Test of significance was done using the Mann-Whitney test, Fisher Exact, and McNeman square test.

### RESULT

**Table 1. Comparison of cyclin D1 expression in patients with hydatidiform mole and normal placenta.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cyclin D1</th>
<th>Category</th>
<th>Control (n=15)</th>
<th>Value p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mola Hydatiform (n=15)</td>
<td>Mola Hydatiform (n=15)</td>
<td>0.001**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cylin</td>
<td>Positive</td>
<td>2 (6.7)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>13 (43.3)</td>
<td>15 (50)</td>
<td></td>
</tr>
</tbody>
</table>

**Value p < 0.05 Fisher exact test**

Based on statistical tests showed there were significant differences in the expression of cyclin D1 in the two groups. In the study group with hydatidiform mole the obtained expression of cyclin D1 is 2, while in the control group did not obtain the expression of cyclin D1, so it can be said that there were statistically significant differences in cyclin D1 expression between patients with hydatidiform mole and those with normal placenta tissue.

**Table 2. Comparison of Mutant Retinoblastoma expression in patients with hydatidiform mole and those with normal placenta.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mutant Retinoblastoma</th>
<th>Category</th>
<th>Control (n=15)</th>
<th>Value p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mola Hydatiform (n=15)</td>
<td>Mola Hydatiform (n=15)</td>
<td>0.017**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mutant Rb</td>
<td>Positive</td>
<td>6 (20)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>Negative</td>
<td>9 (30.00)</td>
<td>15 (50)</td>
<td></td>
</tr>
</tbody>
</table>

**Value p <0.05 based on Fisher exact test**

Based on statistical tests showed there were significant differences in expression between the two groups of mutant retinoblastoma. In the study group with hydatidiform mole the obtained expression of mutant retinoblastoma is 6, while in the control group was found no expression of retinoblastoma. In this study was found statistically significant differences in expression of mutant retinoblastoma between patients with hydatidiform mole and those with normal placenta tissue. Based on histopathology, the expression of retinoblastoma protein are found in patients with hydatidiform mole which is a mutant retinoblastoma.
Table 3. Comparison of expression of cyclin D1 and Mutant Retinoblastoma in hydatidiform mole

<table>
<thead>
<tr>
<th>Rb</th>
<th>Total</th>
<th>Value p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>1 (6.7)</td>
<td>1 (6.7)</td>
</tr>
<tr>
<td>-</td>
<td>5 (33.3)</td>
<td>8 (53.3)</td>
</tr>
</tbody>
</table>

*p < 0.05 based on test of square McNemar

***DISCUSSION***

Comparison of cyclin D1 expression in patients with hydatidiform mole and in those with normal placenta

Involvement of cyclin D in cell cycle control and growth factors of both normal and malignant cells, makes the cyclin D as an oncogene (wild type). Actually this form are also on normal cells but in this condition the cell is not normal, because there is phosphorylated retinoblastoma that suppress the proliferation. As a result of excessive production of cyclin D, G1 phase duration becomes shorter, resulting in growth regulation of cancer cells. Uncontrolled production of cyclin D affects the amount of cyclin D-CDK4 complexes, leading to excessive proliferation of trophoblast cells into mola hydatidiform.11,12

**Figure 1.** Immunohistochemistry staining of cyclin D1 in normal placenta (400x)

Mas Rizky Research (2009) showed that there was increased expression of cyclin D in hydatidiform mole. Cyclin D cause excessive loss of function of TSG (Tumor Suppressor Gene) on the phase of the cell cycle checkpoint that took place without any control and occurs excessive proliferation.13-15

**Figure 2.** Immunohistochemistry staining of cyclin D1 in hydatidiform mole trophoblast (400x)

Comparison of Mutant Retinoblastoma expression in patients with hydatidiform mole and in those with normal placenta

Retinoblastoma (Rb), a protein that functions as a suppressor protein was first discovered in patients with retinoblastoma. This gene in normal circumstances, binds to the transcription factor E2F, the complex is located on the G0 and G1 phase. Rb-E2F complexes split, regardless E2F-free. E2F transcription factor that escapes becomes active, and is instrumental in stimulating the cell cycle to enter the stage of S phase and E2F transcription factors are also required for DNA replication. The entry of the viral genes may also inactivates retinoblastoma Rb and making a mutant virus infection for example the SV40, adenovirus and viral infection7-12.

If the function of TSG is missing then the cell cycle and cell proliferation will not to be well controlled, and makes an abnormal condition such as hydatidiform mole. Studies on the expression of mutant retinoblastoma and p53 mutant showed an increase in hydatidiform mole, which means that the control mechanisms of trophoblast cells from placental trophoblast tissue can develop into a pregnancy failure, in this case a hydatidiform mole.

**Figure 3.** Immunohistochemistry staining of Retinoblastoma Mutant in Normal Placenta (400x)
Comparison of expression of cyclin D1 and Mutant Retinoblastoma in hydatidiform mole

In normal placenta, cyclin D1 is a dominant gene on the cell cycle so that cells can move on to the next phase of the cycle. However, if the function of cyclin D1 is missing then there is the business of compensation by increasing regulation of cyclin D expression of the other so that the cell cycle can take place. This does not occur in hydatidiform mole, the study by Garnier O stated that there was decreased gene expression of cyclin D1, D2, and D3 in hydatidiform mole, so that the compensation process did not occur and resulted in trophoblast cells turning into hydatidiform mole.

Several studies have shown that the expression of retinoblastoma being increased in hydatidiform mole is a mutant retinoblastoma. Retinoblastoma is usually found in patients with mutant breast cancer, lung cancer, renal cancer, bone cancer.\(^1,16,17\) If the mutant was found in hydatidiform mole it was suspected that the function of retinoblastoma is disappeared so that cell proliferation and cell cycle became unwell controlled well, and created an abnormal condition which was hydatidiform mole. Expression of retinoblastoma mutant showed an increase in hydatidiform mole, which means that the control mechanisms of trophoblast cells from placental trophoblast tissue is not going well. If mutant retinoblastoma expression was not found, the risk of degenerating into the abnormality is low.

Expression differences between them can be proved by examination of immunohistochemical staining intensity with the view shown in trophoblast cell nuclei, which role is suspected of cyclin D wild-type and mutant retinoblastoma, but until now there has been no research that ensures the type of cyclin D and retinoblastoma mutant so that the necessary further tests to determine the type of the two are needed.

![Figure 4. Retinoblastoma Immunohistochemistry Staining in hydatidiform mole trophoblast (400x)](Image)

CONCLUSIONS

Cyclin D1 expression increased in hydatidiform mole trophoblast tissue, expression of mutant retinoblastoma was found increased in hydatidiform mole trophoblast tissue and more dominant than cyclin D1.

REFERENCES