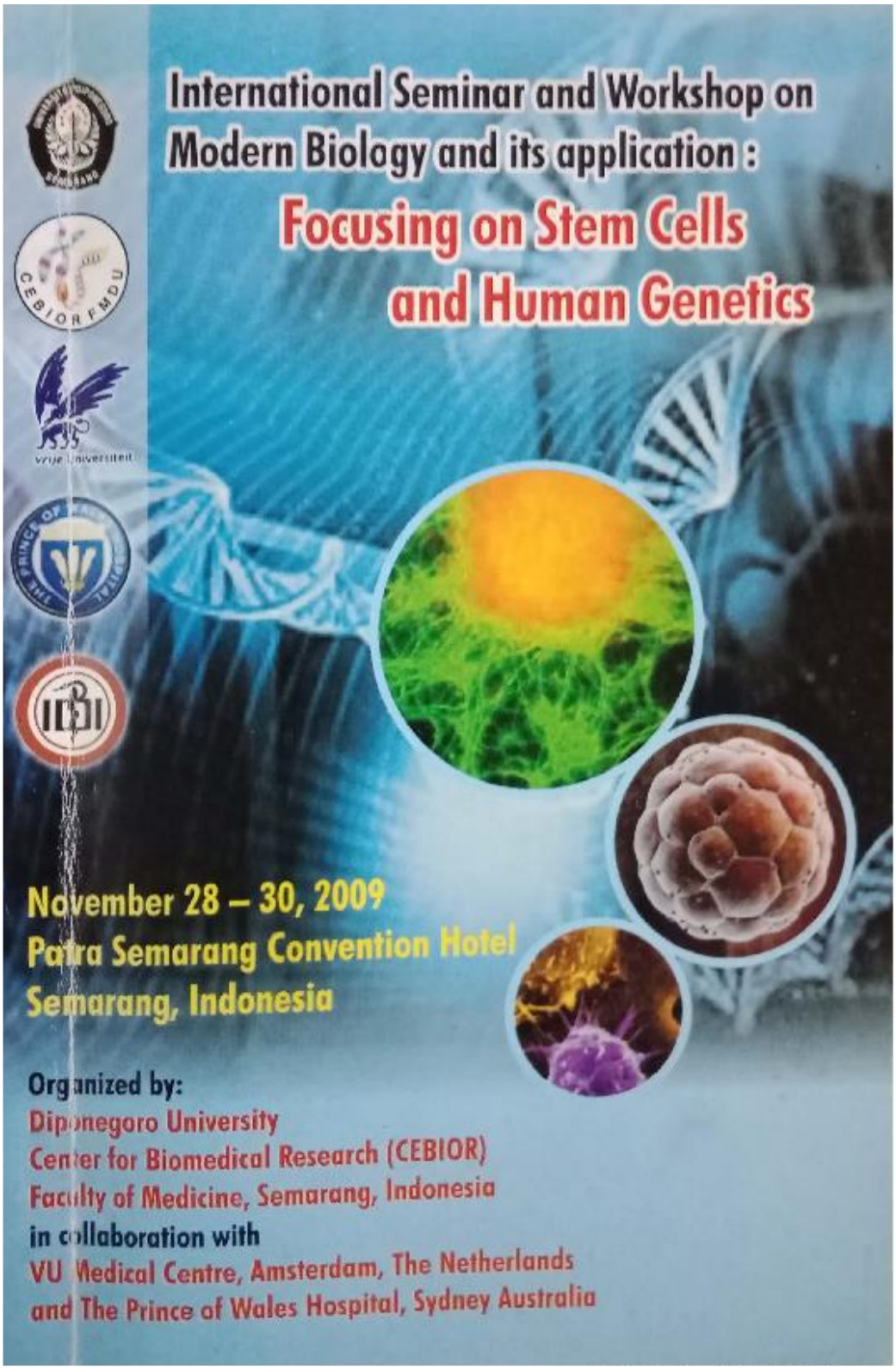
International Seminar and Workshop on
Modern Biology and its application :
Focusing on Stem Cells
and Human Genetics
November 28 – 30, 2009
Patra Semarang Convention Hotel
Semarang, Indonesia
Organized by:
Diponegoro University
Center for Biomedical Research (CEBIOR)
Faculty of Medicine, Semarang, Indonesia
in collaboration with
VU Medical Centre, Amsterdam, The Netherlands
and The Prince of Wales Hospital, Sydney Australia

International Seminar and Workshop on Modern Biology and its Applications: Focusing on Stem Cells and Human Genetics

PROCEEDING BOOK

Editors
Sultana MH Faradz
Annastasia Ediati
Retno Murwani
Yetty Movieta Nency
Neni Susilaningsih

Semarang, Indonesia

November 28-30, 2009

DIPONEGORO UNIVERSITY PRESS
SEMARANG 2009

Published for International Seminar and Workshop on Modern Biology and its Applications: Focusing on Stem Cells and Human Genetics

ISBN 978-979-704-811-2

Cover designed by Eko Adhi Pangarsa

WELCOME REMARKS BY CHAIR

Dear the Rector, Vice Rectors, Deans of 11 Faculties in Diponegoro University, the Board of Directors of Dr. Kariadi Hospital, Mayor of Semarang City, Chairman of IDI (Indonesian Medical Association), invited speakers, participants and ladies and gentlemen,

On behalf of the organizing committee, we would like to extend a warm welcome to all of you in attending this International Seminar and Workshop on Modern Biology and its Applications: Focusing on Stem Cell and Human Genetics.

It is our great pleasure to host all of you here at Diponegoro University especially to our guest speakers from Prince of Wales Hospital/ University of New South Wales, Australia (Prof Robert Lindeman and Dr Annette Tricket), from Vrije University, Amsterdam, the Netherlands (Dr. Erik Sistermans and Dr. Anne-Marie Plass) and from UKM Medical Molecular Biology Institute (UMBI), Universiti Kebangsaan Malaysia (Prof A. Rahman A. Jamal) and all speakers that cannot be mentioned one by one. We would like to thank all of you for taking time to be here.

We designed this event in three parts i.e. Seminar on Molecular Biology, Seminar on Stem Cells, and Workshop on Stem Cells and Modern Biology. We are particularly pleased to see that this event involves many disciplines and from many centers, with the total expected participants about 200 for each seminar, while for the workshop we planned to have limited participants (20 persons for each workshop). We do grateful to see enthusiasm of participants from all over Indonesia (Java, Sumatera, Sulawesi, and Bali) and abroad (the Netherlands, the Philipines, Malaysia, and India) in these events, so there are more than 250 participants registered for seminar, more than 60 participants for stem cell workshop, but we regret to limit on 20 participants for Molecular Biology workshop.

We would also like to thank Rector and Vice Rector for Development and Collaboration and the Dean of Faculty of Medicine for the very enormous supports, since this event is fully funded by the Rector of Diponegoro University in the direction of World Class University (WCU) program accomplishment.

We do appreciate all of the committees and the event organizer who worked hard in preparing this seminar and workshop. We wish you all a productive seminar and workshop. Have a great time in Semarang.

Sincerely,
Sultana MH Faradz
Chair / Director of Center for Biomedical Research (CEBIOR), Faculty of Medicine, Diponegoro University

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Increased Ki-67 expression in complete hydatidiform mole as a prediction factor of choriocarcinoma.

Hana Ratnawati[1], Sunardhi Widyaputra[2] Bethy S Hernowo[2], Silvi Kintawati[2]
[1]Faculty of Medicine, Maranatha Christian University-Bandung,
[2] Master Programs, Faculty of Medicine, Padjadjaran University-Bandung.

Background: The incidence of hydatidiform moles in Indonesia is about 11.6 % per 1000 pregnancies and between 10 % – 50% will develop to choriocarcinoma, a highly malignant neoplasm of trophoblastic cells with poor prognosis. Based on histopathological pattern, complete hydatidiform mole could differentiate to complete mole hydtidiform with less trophoblast proliferation (LTP) and complete hydatidiform mole with high trophoblast proliferation (HTP). Choriocarcinoma usually develop from complete hydatidiform mole as early as 7 days, or it may take up to 3 years to develop, but generally choriocarcinoma begins in the first year. The prognosis depends on the fast treatment on the early stage, that's why it is important to find a biological marker which could predict the development of malignancy in hydatidiform mole. Ki-67 antigen is a nuclear marker of cell proliferation. Several studies showed that a high Ki-67 expession is associated with a greater risk for tumor development.
Objective: To investigate the role of Ki-67 immunoexpression in predicting the malignancy progress of complete hydatidiform mole.
Methods: Formalin fixed, paraffin embedded tissue biopsies from patients with complete hydatidiform moles LPT (n = 15), HPT (n = 17) and choriocarcinoma (n = 15) were studied for Ki-67 expression by immunohistochemistry. Each slide was scored based on the percentage of positively stained malignant nuclei. The following ranges were used for the proliferative index: < 40% (very low), 40% to 60% (low), 61% to 80% (intermediate), and > 80% (high). The data was analyze by ANAVA and Duncans.
Results: The Ki-67 expression of complete hydatidiform mole with LPT, showed very low (46.6%) and low (53.3%) proliferative index, however in complete hydatidiform moles HPT, 29.4% low, 52.9% intermediate and 17.3% very high proliferative index. In choriocarcinoma mostly (86.6%) showed high proliferative index, while only 13.3% with intermediate proliferative index. There was a highly significant difference of proliferative index between complete hydatidiform mole LPT and HPT (p<0.001) and between complete hydatidiform mole LPT with choriocarcinoma (p<0.001).
Conclusion: The high immunoexpression of Ki-67 can predict choriocarcinoma following complete hydatidiform mole with the Odds Ratio 30.33 times.

Key words: complete hydatidiform mole, choriocarcinoma, Ki-67 immunoexpression

Introduction

The incidence of gestational trophoblast disease in Indonesia and developing countries is still high compared to developed countries. The World Health Organization (WHO) reports that the incidence of hydatidiform mole ranges from 1:1,450 to 1:2,000 pregnancies and the incidence of choriocarcinoma 1:14,000 to 1:40,000 pregnancies while in Indonesia, the incidence of moles is 1:51 to 141 pregnancies in West Java. 1:28 to 1:105 pregnancies. (Martaadisoebrata, 2005; Soper, 2006). The thing to be aware is that post-hydatidiform mole malignancy ranges from 15–20%. Post-mole malignancy is growing very fast, with relatively high mortality, 31–51%. The occurrence of post-hydatidiform mole malignancy known as choriocarcinoma is very common, but the method for early detection is not known. The prognosis of choriocarcinoma is worsened and more expensive compared with hydatidiform mole. Therefore, it is essential to evaluate patients with hydatidiform mole complete, which will develop into malignant as early as possible. (Khrismawan, 2004; Soper, 2006).

The incidence of hydatidiform mole in RSHS Bandung 1 : 94 deliveries. In Hasan Sadikin General Hospital (RSHS), complete hydatidiform moles are classified into complete mole hydtidiform with less trophoblast proliferation (CHM/LTP) and complete hydatidiform mole with high trophoblast proliferation (CHM/HTP). (Martaadisoebrata, 2005). The high incidence of hydatidiform mole in Indonesia can be a problem since 10 % – 50% will develop to choriocarcinoma, a highly malignant neoplasm of trophoblastic cells with poor prognosis, even one study found that 69.6 - 82.5% of choriocarcinomas originated from hydatidiform moles. The probability of choriocarcinoma after a complete mole was 4.3 times, especially after CHM/LPT with the mortality rate of Choriocarcinoma is about 30.8% (Martaadisoebrate, 2005). In Hasan Sadikin General Hospital, 54.55% of choriocarcinoma malignancies occurred within four months after the end of pregnancy, therefore early diagnosis and early treatment is important, to avoid malignancy.

Diagnosis of hydatidiform mole is based on clinical symptoms, ultrasound examination, β-hCG levels and a definite diagnosis by histopathological examination. However, these examinations cannot confirm whether a mole has a tendency to become malignant. Studies showed that immunohistochemical techniques can determine the relationship between the proliferative activity of cancer cells and the potential for

malignancy in colorectal carcinoma, and provide a significant prognostic value (Choi et al, 1997). Based on studies in immunohistochemical techniques that have been carried out by previous researchers, in this study will detect the possibility of malignancy post hydatidiform mole. It is important to find a biological marker which could predict the development of malignancy in hydatidiform mole. Various oncogens are thought to be associated with cell proliferation and the expression can describe the tumor progression. One of the specific marker for cell proliferation is Ki-67, a nuclear marker for cell proliferation in which the higher percentage of Ki-67 immunoexpression the more malignant a tumor is. (Guinebretière, 1997).

Methods :

This study was conducted retrospectively on CHM/LTP, CHM/HTP and choriocarcinoma. A total of 47 tissue samples and data were included in this study with 3 subgroups, 15 CHM/LTP, 17 CHM/HTP cases and 15 choriocarcinoma cases. The formalin-fixed paraffin-embedded tissues and data of the patients were collected from the Anatomical Pathology Department Hasan Sadikin General Hospital and from Immanuel Hospital. The hematoxylin and eosin sections were reviewed to select representative areas of the tumour and normal-adjacent tissue.

Immunohistochemical Ki-67 analysis was performed with LSAB™ Kit/HRP. Development of reactions was performed with DAB (Diaminobenzidine tetrahydrochloride). As a control negative used tumor tissue without given primary antibodies and as the positive controls used colon tissue carcinoma with Ki-67 positive. The quantification of reactions was performed independently by two people. Ki-67 immunoexpression assessment is carried out by counting the immuno-reactive tumor cells under a light microscope with a 400 X magnification of the entire mass of the tumor until 100 cells were obtained. Immunoexpression is positive if the nucleus is brown with a purple tissue background. Cells showing immunoreactivity were counted semi-quantitatively as follows:

- Very low if less than 40% of cells are immunoreactive
- Low if 40 - 60% of cells are immunoreactive
- Moderate if 61 - 80% of cells are immunoreactive
- High if more than 80% of cells are immunoreactive

Statistical analysis used ANAVA and Duncans by SPSS software.

Resuls and Discussion

This study included 32 complete hydtaidiform mole with 15 cases less trophoblastic proliferation and 17 cases with highly trophoblastic proliferation and 15 choriocarcinoma cases. The characteristic of the research subjects described in table 1.

Table 1. Age distribution of the research subjects.

Age (yrs)	Complete Hydatidiform		Choriocarcinoma (cases)
	LTP (cases)	HTP (cases)	
< 20	2	2	-
20 - 29	11	8	4
30 – 39	2	3	2
>40	-	4	9
Total	15	17	15
Average age	24.5	29.3	37.4

Table 1 showed that in CHM/LTP and CHM/HTP, the most cases were in the age range between 20-29 years with 11 cases (73.3%) CHM/LTP and 8 cases (47%) CHF/HTP. The mean age of patients with CHM/LTP mole was quite young, 24.5 years, while the mean age of CHM/HTP was 29.3 years. Most cases of choriocarcinoma were found at age more than 40 years with 9 cases (60%), with a mean age of 37.4 years. There were 4 choriocarcinoma cases with age less than 30 years, a very young age for a malignant disease, that is why early diagnose is important in complete hydatidiform mole patients with tendency to become malignant.

This result is almost the same as reported by Bratakoesoema (1996) who found 64.1% of patients were in the 20-29 years age group, and only 7.6% more than 35 years old. The highest incidence of post molar trophoblast tumors was found in the 35 - 39 years age group. Meanwhile, the other researcher found that the incidence of choriocarcinoma at age more than 45 years was 86.9% and 83.3% of cases occurred

post molar. In this study, 60% incidence of choriocarcinoma was found at age more than 40 years. It means that the incidence of choriocarcinoma at reproductive age is still high, if early diagnosis can be confirmed, it will improve the prognosis of post molar patients. Immunoexpression of Ki-67 is a marker for cell proliferation, hopefully can predict the progression of complete mole become a malignant tumor. The immunoexpression of Ki-67 showed in Table 2.

Table 2. Ki-67 Immunoexpression in Complete Hydatidiform Mole and Choriocarcinoma.

Ki-67 immuno-expression (%)	**Complete Hydatidiform**		**Choriocarcinoma**
	LTP	**HTP**	
Very low (<40)	7 (46,6 %)	-	-
Low (40 – 60)	8 (53,3 %)	5 (29,4 %)	-
Moderate (60 – 80)	-	9 (52,9 %)	2 (13,3 %)
High (> 80)	-	3 (17,6 %)	13 (86,6 %)
	15 (100%)	17 (100%)	15 (100%)

LTP : Less Trophoblastic Proliferation
HTP: Highly Trophoblastic Proliferation

Immunoexpression of Ki-67 in CHM/LTP range from very low (46.6%) to low (53.3%) group. In CHM/HTP 52.9% in moderate group, while in the choriocarcinoma 86.6% of cases were in the high group. The level of this proliferation is in accordance with the histological description. In the CHM/HTP group, there were 3 cases (17.6%) in the high group, it was case number 4 (age 24 years) with Ki-67 immuno-expression 81%, case number 14 (age 40 years) with Ki-67 immuno-expression 85% and case number 16 (age 45 years) with Ki-67 immuno-expression 83%. The more malignant a tumor is, the higher the percentage of Ki-67 immunoexpression (Guinebretière, 1997; Cheville, 1996).

Table 3. Correlation of Ki-67 immuno-expression between choriocarcinoma and CHM/HTP

Ki-67 immunoexpression	CHM/HTP (cases)	Choriocarcinoma (cases)	%
High	3	13	23.1
Moderate - Low	14	2	14.3
Total	**17**	**15**	**37.4**
	OR = 30.33		**p<0.001**

To determine the progression possibility of CHM/HTP towards choriocarcinoma, the Ki-67 immunoexpression analyzed with Fisher's exact test and the result showed in table 3. It was found that the Ki-67 immuno-expression in CHM/HTP was high in case no. 4, 14 and 16 and were highly significant ($p < 0.001$) for the occurrence of choriocarcinoma and the odds ratio showed that the risk of choriocarcinoma was 30.33 times.

There were 3 cases in CHM/HTP that showed high Ki-67 immuno-expression, more than 80%. High Ki-67 immunoexpression in CHM/HTP was highly significant in predicting the occurrence of choriocarcinoma and the chance for the occurrence of choriocarcinoma is 30.33 times. This was also reinforced by the results of the morphological analysis of trophoblast cells in which, Ki-67 immunoexpression was mainly in cytotrophoblast cells and trophoblast intermediates. If the intermediate cells showed high Ki-67 immunoexpression, it is due to the function of those cells to produce hCG and it means that the proliferative activity will increased.(Cheung, 1998; Ichikawa, 1998). The production of hCG per one trophoblast cell is 10-4 to 10-5 IU hCG. Thus a complete mole, which shows a high Ki-67 immunoexpression score and in the syncytiotroblast cells and trophoblast intermediates, will have a worse prognosis. (Ostrzega, 1998; Schammel, 1996). This is in accordance with the results of research by Bratakoesoema (1996) which states that the activity of trophoblast cells plays a major role in the occurrence of post-molar gestational trophoblast tumors.

Conclusion

The highest incidence of complete hydatidiform mole is in 20-29 years, wherease choriocarcinomas above 40 years.

The high immunoexpression of Ki-67 can predict choriocarcinoma following complete hydatidiform mole and the ODD's ratio is 30.33 times.

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