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Correlation between Three Pregnancy Characteristics (Age, Parity, βhCG Level) and pAkt Immunoexpression on Complete Hydatidiform Mole

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Abstract

The incidence of hydatidiform mole as pregnancy failure is still high in Asia, leading to high mortality and morbidity. Several risk factors play roles in the occurrence of hydatidiform mole, including age and parity. Until now, β-human chronic gonadotropin (βhCG) is used to predict the risk and development of hydatidiform mole. Akt, also known as protein kinase B (PKB), is a downstream effector of the intracellular phosphatidylinositol 3-kinase (PI3K) signaling pathway, which is a central regulatory pathway for cell proliferation, growth, differentiation, and survival. This study aimed to analyze the correlation between three pregnancy characteristics *i.e.* age, parity, levels of βhCG and pAkt immunoexpression. Cross sectional study was conducted on 30 samples of complete hydatidiform mole (CHM). The βhCG content was measured by ELISA method. The immunoexpression of pAkt was measured by immunohistochemical staining using the phospho-Akt antibody (Ser473) (736E11) Rabbit mAb # 3787 (CST). Cells with positive pAkt immunoexpression showed brown color. The stronger the intensity of the visible brown color, the higher the level of expression. pAkt immunoexpression level was then expressed as histoscore value, which was calculated using staining intensity and positively-stained-cell distribution number. When the immunoexpression level was high, its histoscore value would be high too. Results of this study showed that the histoscore of pAkt samples ranged from 0 - 16 with a median value 4, and the most samples (56.7%) had histoscore ≤ 4. Correlation coefficient (p) value that was used in this study was 0.01. Correlation between two variables was significant if $p < 0.01$. Correlation coefficient (p) value between age and pAkt immunoexpression was 0.260 and thus was not sta-

tistically significant, neither was the number of parity and pAkt immunoexpression ($p = 0.524$). However, βhCG level and pAkt immunoexpression showed statistically significant correlation ($p = 0.00$). It was concluded that there was no significant correlation between maternal age and parity and pAkt immunoxpression ($p > 0.01$), but there was a significant correlation between βhCG levels and pAkt immunoxpression ($p < 0.01$).

Subject Areas

Gynecology & Obstetrics

Keywords

Complete Hydatidiform Mole, Akt, βhCG, Age, Parity

1. Introduction

There are two types of hydatidiform mole as pregnancy failure, *i.e.* complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM), which are cytogenetically different. CHM exhibits androgenetic properties, where all chromosomes in CHM tissue are derived from paternal cells. CHM pregnancy occurs because an empty ovum is fertilized by a 23x haploid sperm, resulting in conception with 23x chromosome. This chromosome then undergoes self doubling (endoreduplication) to 46xx. PHM is generally triploid or trisomic on a single chromosome. In CHM, all chorionic villi are hydropic degenerated so that no fetal element is found, whereas in PHM there is fetus, umbilical cord, normal amniotic membrane, and placenta with hydropic degenerated chorionic villi [1] [2] [3] [4].

The incidence of hydatidiform mole in the world varies depending on the region, differences can even be found in the regions of the same continent. Based on the report of The National Coordination Research Group of Chorioma (NCRG) hydatidiform mole incidence in China was 0.78 per 1000 live births. The incidence of hydatidiform mole in Japan was much higher, reaching 2.0 per 1000 live births, 3 times higher than the incidence in European or North American countries (0.6 - 1.1 per 1000 pregnancies). Other studies showed that hydatidiform mole incidence in Japan ranged from 2.83 to 3.05 per 1000 live births. The highest incidence of hydatidiform mole was reported in Indonesia, *i.e.* 1 in 77 pregnancies or 1 in 57 deliveries [5]. Martaadisoebrata *et al.* in Hasan Sadikin Hospital Bandung, Indonesia, showed that the incidences of gestational trophoblast disease over three periods (1971-1976, 1996-2000, 2001-2005) were still high (19.8, 30.8, 23.6 per 1000 births), and the percentages of hydatidiform mole were 83%, 67%, and 45% respectively [6].

Some risk factors are known to play a role in the occurrence of hydatidiform mole, *i.e.* maternal age, parity, race, malnutrition, environmental factors, previous hydatidiform mole history, consanguinity, and genetics [1] [5]. Age groups

with higher risk for hydatidiform mole are those who are pregnant at under 20 and over 35 years old. Within the age groups above 40 years old, hydatidiform mole incidence is known 4 - 10 times higher than those at age 20 - 40 years old.

In addition to age, parity can be a risk factor for hydatidiform mole, although some studies showed different results. A study in Italy showed an increased risk of hydatidiform mole in nullipara with miscarriage history. However, other studies that were also conducted in Italy and the Rhode islands, United States, showed that parity was not associated with hydatidiform mole risk [5]. Martaadisoebrata [7] in Bandung demonstrated a positive correlation between parity and hydatidiform mole incidence. Several other studies found an association between parity and hydatidiform mole development to gestational trophoblast tumor. Aziz *et al*. [8], Khrismawan *et al*., [9] and Prajatmo [10] found a significant correlation between parity and the incidence of post-CHM gestational trophoblast tumor malignancy. In contrast to these studies, Yudi *et al*. showed no significant relationship between parity and hydatidiform mole in persistent CHM cases and CHM cases with spontaneous regression [11].

Hydatidiform mole causes high morbidity and mortality. Complications that often accompany hydatidiform mole may be early complications such as bleeding, preeclampsia, hyperthyroidism, and thyrotoxicosis, or advanced complications such as gestational trophoblast tumor [3]. Therefore, early detection of CHM is essential to prevent the onset of these complications.

Until now, the level of βhCG has been widely used to predict the risk and development of hydatidiform mole [12] [13]. βhCG level is also used as a fundamental consideration for treatment, both prophylaxis and definitive therapy for gestational trophoblast tumor [3] [4] [13]. βhCG level > 100,000 mIU/ mL indicates a high risk of hydatidiform mole, hence some treatment centers establish prophylactic chemotherapy [3] [4] [13]. Monitoring of post-evacuation serum βhCG level in CHM patient management guidelines at Hasan Sadikin Hospital Bandung was performed using Mochizuki regression curve [14]. Evaluation of serum βhCG level is performed at week 2, 4, 6, 8, and 12 post-evacuation of CHM. Deviation of the normal regression curve or the distortion toward elevated serum βhCG level indicates persistent trophoblastic diseases or transformation of post-CHM malignancy into gestational trophoblast tumor [14].

βhCG is a subunit of human chorionic gonadotropin (hCG) hormone, a glycoprotein consisting of two subunits, *i.e.* α subunit (identical to α subunits of LH, FSH and TSH glycoprotein hormones) and β subunits (specific to hCG) with 8 sugar chains. The α subunit has 92 amino acids, while the β subunit has 145 amino acids. Based on composition of these structures, there are several variants of hCG hormone. The four major variants of hCG structure commonly detected in serum are hCG free β subunit, nicked hCG, hCG missing the β subunit C-terminal peptide, and hyperglycosylated hCG. Measurement of βhCG level is a common examination to assess the presence of hCG hormones in serum, urine, or other body fluids [12].

hCG is produced by trophoblast cells especially syncytiotrophoblasts, so that

the excessive proliferation of syncytiotrophoblasts will increase hCG level [12] [15]. In CHM pregnancy, the proliferation rate of trophoblast cells, both syncytiotrophoblast and cytotrophoblast, is higher than in normal pregnancy, hence hCG level in CHM pregnancy is generally higher than in normal pregnancy [1] [2]. During pregnancy, hCG stimulates corpus luteum cells to produce progesterone in order to maintain pregnancy. hCG is luteotropic hormone and corpus luteum has a high-affinity receptor for hCG. Thus, under conditions of very high hCG level, cysts are frequently found in corpus luteum [3] [11].

Akt or protein kinase B (PKB) is a downstream effector of the intracellular phosphatidylinositol 3-kinase (PI3K) signaling pathway, which is the central regulatory pathway for cell proliferation, growth, differentiation, and survival [16] [17]. Akt will convey the intracellular transduction signals stimulated by growth factors such as platelet-derived growth factor (PDGF), epidermal growth factor (EGF), basic fibroblast growth factor (BFGF), and insulin-like growth factor (IGF-I), which will then activate thousands of downstream substances. Akt activity will change the subcellular location or modify the stability of protein, which further affects the activity of downstream substances in regulating cell proliferation, differentiation, invasion, and apoptosis [16].

In trophoblast cells, Akt regulates the phenotype differentiation of the cells. Akt also plays role in coding proteins that potentially cause direct trophoblast invasion to maternal/ uterine environment (MMP9, IGF2, Serpine1), affecting immunity and vascular cells (Cgm4, Faslg), regulating androgen biosynthesis, and activating trophoblast invasion process [17] [18].

Choi *et al*. showed an association between Akt and age on rats. Akt plays role in FoxO3a phosphorylation, a transcription factor that plays an important role in aging processes. FoxO3a's activity decreases with age, and negatively regulated through phosphorylation by PI3K/Akt signaling pathway [19]. Prast *et al*. showed that hCG increases the phosphorylation of Akt so that Akt becomes active and phosphorylates the downstream substances [15].

This study aimed to analyze the correlation between demographic characteristics: age, parity, βhCG levels and pAkt in CHM pregnancy.

2. Methods

This was a cross sectional study on 30 patients that were diagnosed with CHM based on the examination results conducted at Anatomy & Pathology Laboratory of Hasan Sadikin Hospital, Faculty of Medicine, Padjadjaran University. The CHM patients were selected based on sample criteria that were recorded in the last five years at Obstetrics and Gynecology Department and Anatomy & Pathology Laboratory of Hasan Sadikin Hospital, Faculty of Medicine, Padjadjaran University. Ethical clearance for this study was obtained from Health Research Ethics Committee, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia (Registration number: 0515020111).

βhCG levels in CHM patient tissues were measured by ELISA method. pAkt immunoexpression was measured by immunohistochemical staining using

phospho-Akt antibody (Ser473) (736E11) Rabbit mAb # 3787 (CST). The research was performed at Anatomy & Pathology Laboratory of Hasan Sadikin Hospital, Faculty of Medicine, Padjadjaran University, Bandung, Indonesia. Immunoexpression assessment of pAkt was determined based on staining intensity and cell distribution as described in Table 1.

The histoscore from the staining results were calculated using following formula: [20]

$$\text{Histoscore} = \sum (i+1) P_i$$

i: intensity

P_i: cell distribution with positive staining

Data were then analyzed statistically using chi square analysis, with correlation coefficient (p) value 0.01. Correlation between two variables was significant if $p < 0.01$.

3. Results

A total of 30 CHM cases were collected in this study. Data collection on demographic characteristics (maternal age and parity) and βhCG levels were conducted. Immunohistochemical staining was then performed to assess the pAkt immunoexpression. Histoscore calculation of pAkt immunoexpression was applied on all subjects. Immunoexpression assessment of pAkt was determined based on staining intensity and cell distribution.

Table 2 shows that most of patients involved in the study were in the age of 20 - 34 years old which are reproductive age. This can happen because most pregnancies occur at that range of age.

From 30 examined CHM samples, histoscore values ranged from 0 - 16 with median 4. Most samples (56.7%) had histoskor values ≤ 4.

Cells with positive pAkt immunoexpression showed brown color. The stronger the intensity of the visible brown color, the higher the level of expression (Figure 1).

Table 3 shows no significant correlation between age and parity and pAkt immunoxpression ($p > 0.01$). However, βhCG level and pAkt immunoexpression

Table 1. Immunohistochemical scores determination.

Color Intensity	Score
No cells are stained brown	0
Weak staining intensity: light brown	1
Intermediate staining intensity: brown	2
Strong staining intensity: dark brown	3
Cell Distribution	**Score**
<20%	1
20% - 50%	2
50% - 80%	3
≥80%	4

Table 2. Distribution table of the results.

Characteristics		Number	Percentage (%)
Age	<20	3	**1**
	20 - 34	16	**53.3**
	35 - 39	4	**13.3**
	≥40	7	**23.3**
Parity	0	9	**30**
	1 - 2	11	**36.7**
	≥3	10	**33.3**
βhCG Level	<100,000	13	**43.3**
	≥100,000	17	**56.7**
pAkt Histoscore	Range	**0 - 16**	
	Median	**4**	

Table 3. Correlation between demographic factors (Age, parity, βhCG Level) and pAkt immunoxpression.

Demographic Factors		pAkt Immunoxpression (Histoscore)		p value
		≤4	>4	
Age	<20	1	2	
	20 - 34	8	8	0.260
	35 - 39	4	0	
	≥40	4	3	
Parity	0	4	5	
	1 - 2	6	5	0.524
	≥3	7	3	
βhCG Level	<100,000	13	0	0.00
	≥100,000	4	13	

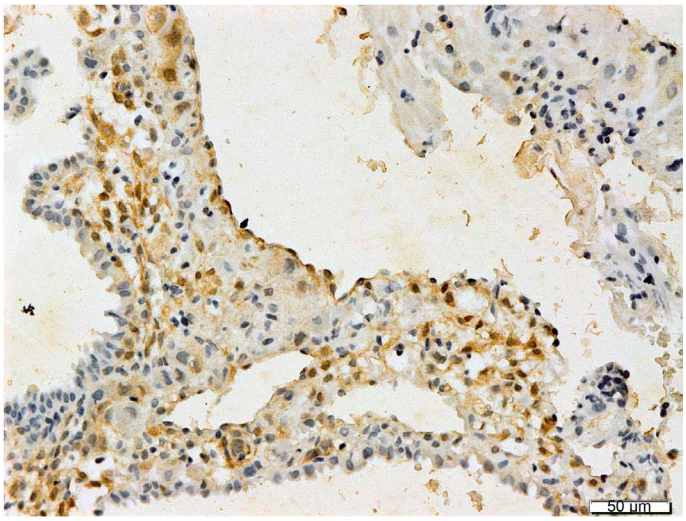

Figure 1. pAkt immunohistochemical staining on CHM Tissues.

was significantly correlated ($p < 0.01$).

4. Discussion

1) Correlation between Age and Parity and pAkt Immunoexpression

It is known that age is one of the important risk factors in hydatidiform mole incidence as well as the transformation of post-hydatidiform mole malignancies. The incidence of CHM increases at extreme age (under 20 years, and over 35 years). There is a progressive increase in risk (more than 10 times) in women over the age of 40. Prajatmo showed that the age group ≥35 years old had 2 times greater risk when compared with age <35 years. [10] Theoretically, at that age, the risk of sex chromosome division (X chromosome) failure in ovum meiogenesis is higher. Failure in the process can produce ovum without X chromosome (empty ovum). Fertilization on the empty ovum will produce an abnormal zygote with sex chromosomes that only originate from the father (paternal/spermatozoa). Without maternal sex chromosome, trophoblast cells of the zygote will undergo self reduplication *i.e.* endoreduplication, which is the pathogenesis of CHM (Androgenetic theory) [4].

Similarly, previous epidemiological studies conducted by Martaadisoebrata *et al*. [7] in Bandung showed a positive correlation between parity and hydatidiform mole incidence. In pregnancy, trophoblast cells always circulate in the blood circulation throughout mother's body, hence an immunological reaction between trophoblast cells and immune system occurs in mother's body. Under these conditions, the shorter the time of the pregnancy interval, mother's immunologic reaction disorder will more likely increase, thus increasing the incidence of hydatidiform mole pregnancy in mother with high parity [19].

Akt plays role in FoxO3a phosphorylation, a transcription factor that plays an important role in the aging process. FoxO3a expression decreases with age, and are negatively regulated through phosphorylation of PI3K/Akt signaling pathway. The expression of Akt itself increases with age. Choi *et al*. [19] showed that ferulate administration in rats can suppress Akt activity, and thus inhibit the aging process.

However, the results of this study indicated that age was not statistically correlated with pAkt immunoexpression ($p > 0.01$). This might happen because in CHM the relationship between Akt expression and age does not follow the usual pattern of normal cells, yet this suposition needs to be investigated further. The same presumption can be applied for parity factor. Age and parity are generally linearly related, where the older women usually have higher parity. Therefore, this study also showed no significant correlation between parity with pAkt immunoexpression ($p > 0.01$).

2) Correlation between βhCG Level and pAkt Immunoexpression

βhCG is produced by trophoblast cells, especially syncytiotrophoblasts. Due to excessive trophoblast proliferation, hCG will be produced more. Until now, βhCG level is a highly important variable for diagnosis, monitoring of malignant development, and evaluation of treatment success in gestational trophoblast

disease case [3] [13].

In CHM, the proliferated trophoblast cells fill uterine cavity. Along with the increased proliferation rate of trophoblast cells, especially syncytiotrophoblasts, serum βhCG level will tend to increase, and potentially reach above 5,000,000 mIU/mL. High level of βhCG is an indicator of high trophoblast activity. Experts agree that elevated level of βhCG can be used as specific and sensitive marker to predict CHM post-evacuation malignancy [13]. FIGO, ISSTD, and WHO established pre-evacuation serum βhCG level ≥100,000 mIU/ mL as a parameter that indicates a high risk of CHM transformation into malignancy [13]. Martaadisoebrata, [7] Khrismawan *et al*. [9], and Pradjatmo [10] obtained similar results that pre-evacuation βhCG serum level ≥100,000 mIU/mL was a risk factor for CHM post-evacuation malignancy.

This study showed significant correlation between βhCG level and pAkt immunoexpression ($p < 0.01$). The high value of pAkt histoscore was associated with high level of βhCG. This finding was in line with previous research showing that Akt will be activated/phosphorylated by hCG administration. Thus, the higher βhCG level, the higher pAkt expression [15]. It has been known that hCG has a synergistic effect on PI3K-Akt, ERK, and MAPK signalling pathways against cytotrophoblast invasion [16].

5. Conclusion

From the results obtained in this study, it was concluded that maternal age and parity were not significantly correlated with pAkt immunoxpression ($p > 0.01$). However, there was a significant correlation between βhCG levels and pAkt immunoxpression ($p < 0.01$).

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