Research Article

Bax Expression of Throphoblast Cells did not Differ between Early and Late Onset Preeclampsia

Ekspresi Baks Sel Trofoblas tidak Berbeda antara Preeklamsia Awitan Dini dan Lanjut

Made Ariyana, Diah R. Hadiati, Irwan T. Rachman, Dewajani Purnomosari

Department of Obstetrics and Gynecology
Faculty of Medicine Universitas Gadjah Mada
Dr. Sardjito General Hospital
Yogyakarta

Abstract

Objective: To compare Bax protein expression in throphoblast cells of early and late onset PE.

Methods: A cross sectional study involving 36 cases of early onset PE and 36 cases of late onset PE was conducted. Bax protein expression was evaluated from sample of placental tissue collected from the study population and calculated using H-Score. Data on age, number of parity, gestational age, body mass index was collected from the medical records. Expression of Bax was compared using Mann-Whitney test.

Result: There was no difference in the clinical characteristics (age, number of parity, BMI, SBP, DBP, and MAP) between the two groups. There was no difference in the expression of Bax protein between the early and late onset PE (mean H-score early vs. late onset PE: 1.48 vs 1.46, p=0.814, Mann Whitney U test). Clinical characteristics of the study population also did not correlate with the Bax expression (R for number of parity: 0.052, age: 0.009, gestational age: -0.014, BMI: 0.063, all p values were >0.05, linear regresion).

Conclusions: There is no difference in the expression of Bax protein of throphoblast cells between early and late onset PE.

Keywords: apoptosis, BAX, early onset, late onset, preeclampsia.

Abstrak

Tujuan: *Untuk membandingkan ekspresi protein Baks dalam sel trofoblas pada preeklamsia (PE) onset dini dan lambat.*

Metode: *Sebuah studi potong lintang yang melibatkan 36 kasus PE onset dini dan 36 kasus PE onset lambat dilakukan. Ekspresi protein Baks dievaluasi dari sampel jaringan plasenta yang dikumpulkan dari populasi studi dan dihitung menggunakan skor-H. Data usia, jumlah paritas, usia kehamilan, indeks massa tubuh dikumpulkan dari rekam medis. Ekspresi Baks dibandingkan menggunakan uji Mann-Whitney.*

Hasil: *Tidak terdapat perbedaan pada karakteristik klinis (usia, jumlah paritas, IMT, TDS, TDD, dan MAP) antara kedua kelompok. Tidak terdapat perbedaan dalam ekspresi protein Bax antara PE onset dini dan lambat (rata-rata H-skor PE onset dini dan lambat: 1.48 vs 1.46, p = 0.814, uji Mann Whitney U). Karakteristik klinis populasi studi juga tidak berkorelasi dengan ekspresi Bax (R untuk jumlah paritas: 0,052, usia: 0,009, usia kehamilan: -0,014, BMI: 0,063, nilai p dari semua variable tersebut adalah sebesar >0,05, dengan menggunakan regresi linier).*

Kesimpulan: *Tidak terdapat perbedaan dalam ekspresi protein Baks pada sel trofoblas antara PE onset dini dan lambat.*

Kata kunci: *dapoptosis, baks onset dini, onset lambat, preeklamsia.*

Correspondence author. Made Ariyana. Department of Obstetrics and Gynecology Faculty of Medicine Universitas Gadjah Mada. Dr. Sardjito General Hospital. Yogyakarta ariyana5151@gmail.com

Received: August, 2020 Accepted: June, 2021 Published: July, 2021

INTRODUCTION

Preeclampsia (PE) is a multisystem pathology of pregnancys characterized by the development of hypertension and its clinical consequences after 20 weeks of gestational age. PE is still notorious for its high maternal and perinatal mortality. PE is a direct cause of maternal mortality worldwide which associated with severe complications (i.e. intracerebral bleeding, pulmonary edema, heart and renal failure). The worldwide incidence of PE varies between 3-5%. Annualy, there were approximately 500,000 maternal and 900,000 perinatal deaths associated with PE in developing countries.[1-3] The annual incidence of PE in Indonesia is even higher, i.e. between 5-10%. Most importantly, the rate tends to increase year to year.

The exact mechanisms that underlie PE are still elusive. Hence, the treatment for PE is still mainly symptomatic. Several treatment modalities might reduce the risks for complication but the firm evidences assuring its safety for the mother and child are still lacking. Due to its elusive patho-mechanism, the effective prevention and treatment for PE is yet to be discovered. However, many experts believe that PE result from pathologic process that develops within the placenta. For example, the role of placental hypoxia that result from inadequate cytotrophoblasts invasion into the spiral arteries or inadequate spiral arterial remodeling that subsequently induce oxidative stress and endothelial dysfunction. The placenta in pregnancy with PE is suspected to be the source of oxidative stress and hence, the free radicals.[4] The apoptotic activity of the throphoblast may also play a crucial role in the development of PE, particularly the onset of development.

Therefore, this study is aimed to evaluate the expression of proapoptotic protein Bax within the throphoblast cells and compare it between early and late onset PE.

METHODS

This is a cross sectional study that involved singleton live pregnancy aged 20-40 weeks with early and late PE as case and control groups, respectively. The study was conducted at the Emergency Maternal Ward, Department of Obstetrics and Gynecology Dr. Sardjito General Hospital Yogyakarta from Mei 2020 to July 2020. The eligible subject who meet the inclusion criteria was recruited into the study population and a 3x3 cm placental tissue was sampled following delivery. Data were analyzed using SPSS for Windows version 24.

Bax protein expression was examined using immunohistochemistry. Bax protein expression was evaluated within the decidual thophoblast cells. HSCORE was calculated using the formula ΣPi x (i+1), in which *Pi* was the percentage of positive cells, i was the intensity of staining with value 0 for negative staining, for weak staining, for moderate staining, and for strong staining. H-Score was evaluated by three independent observers who were not aware about the identity and diagnosis of the sample. Inter-observer validation was done using intra-class correlation (r). Data on age, gestational age, parity and BMI were obtained from the medical records. Age, gestational age, number of parity, BMI and H-Score were analyzed for their normality using Shappiro-Wilk test. Mann-Whitney test was utilized to determine the difference of Bax protein expression between early and late PE.

This study has been approved for ethical eligibility from the Research Ethics Committee of Medical Faculty of Universitas Gadjah Mada / Dr. Sardjito General Hospital Yogyakarta Protocol Number KE/0703/07/2020.

RESULTS

Table 1 summarizes the characteristics of the study population. There was no difference in the age, number of parity, BMI, SBP, DBP, and MAP between the two groups.

Table 1. Baseline Characteristics of the Study Population

Variables	Early onset PE	Late onset PE	*P-value*
	Mean ± SD	Mean ± SD	
Age (years)	30.3 ± 4.9	30.6 ± 6.9	0.473†
Number of parity	0.6 ± 0.7	0.7 ± 0.8	1.000†
Gestational age (weeks)	31.1 ± 2.2	37.2 ± 1.9	0.000†
BMI (kg/m2)	26.8 ± 4.7	27.6 ± 5.8	0.506*
Systolic BP (mmHg)	167.1 ± 20.2	166.4 ± 18.0	0.878*
Diastolic BP (mmHg)	99.7 ± 11.4	103.1 ± 12.4	1.000†
MAP (mmHg)	122.1 ± 12.9	124 ± 13.1	0.503*

**T-independent test, normally distributed data. † Mann-Whitney U test, non-normally distributed data.*

Expression of Bax protein of throphoblast cells

Figure 1 represent Bax expression observed within the selected specimens. There was no difference in the expression of Bax protein between the early and late onset PE (Table 2). Clinical characterictis of the study population did not correlate with the Bax expression (Table 3).

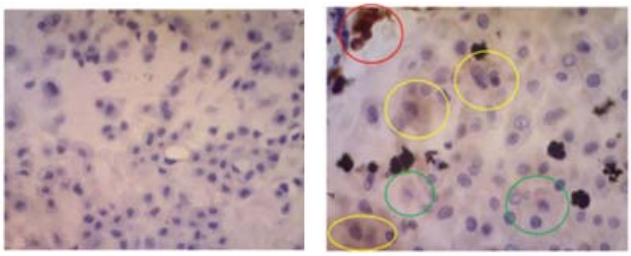

Figure 1. Left. No staining or negative staining. Right. Variable staining depicting strong (red circle), moderate (yellow circle), and weak staining (green circle). 400x magnification, light microscopy.

Table 2. Comparison of Bax Expression between Early and Late Onset PE.

PE	N	Mean H-Score	SD	*P-value*
Early	36	1.48	0.48	0.48
Late	36	1.46	0.46	0.46

Mann-Whitney U test

Table 3. Correlation between Baseline Characteristic and Bax Expression

Characteristic	R	*P-value*
Number of parity	0.052	0.332
Age	0.009	0.469
Gestational age	-0.014	0.453
BMI	0.063	0.299

DISCUSSION

PE can occur ealier in pregnancy (less than 34 weeks) and hence, called early-onset PE, or later than 34 weeks of pregnancy. The difference in the onset may be attributed to the difference in invasive capacity of cytotrophoblasts into the spiral arteries that begin in the first trimester (16-18 weeks). Late-onset PE is more common than the ealy-onset PE (2.7%-88% vs. 0.38% - 12%).[5] The influence of maternal age on the risk of PE is still controversial. In developed countries, advance maternal age (older than 35 years) has been associated with the increased risk of pregnancy complications such as abortion, fetal demise, gestational hypertension and PE).

In this study, the clinical characteristics did not differ between the two groups. Similar result also reported, in which there were no difference of mean age between early and late PE (30.3 ± 4.9 vs. 30.6 ± 6.9, p = 0.473).[6] Early onset PE tend to occur in older age group, while late onset PE was associated with chronic hypertension.[7] Low number of parity was associated with the earlier onset of PE, while multiparity was associated with the later onset of PE. Nulliparity has been cited as one the risk factors for the development of PE.[8], The risk for PE in nulliparas was 1.1 (0.73-1.66) but the association was not statistically significant (p = 0.657).[10] No significant association between number of parity and the risk of PE.[11] Our study also reporting similar result in which no significant difference in the number of parity between the two groups.

In this study, we demonstrate no difference in the expression of Bax protein between early and late onset PE. Similar result also demonstrated in which Bax expression did not differ between early and late onset PE. Immunohistochemistry offers significant advantage for Bax protein characterization since it can reflect the direct event of apoptosis in the placenta.[12] It is practically and ethically difficult to evaluate Bax protein expression on the placenta to predict the future emergence of preeclampsia. However, results from this study provide important information and serve as a basic theory to further evaluate the association of Bax levels in the placenta and the development of PE.

CONCLUSION

We conclude that the expression of Bax protein in the throphoblast cells did not differ between early and late onset PE. We recommend further study that use more accurate evaluation of Bax expression such as radioimmunoassay of Western ligand blotting.

REFERENCES

1. Gupta Sajal, Aziz Nabil, Sekhon Lucky, et al. Lipid peroxidation and antioxidant status in preeclampsia: a systematic review. Obstet Gynecol Survey. 2009; 64 (11): 750-9. doi: 10.1097/OGX.0b013e3181bea0ac.
2. Powe, Camille E.; Levine, Richard J.; Karumanchi, S. Ananth. Preeclampsia, a disease of the maternal endothelium: the role of antiangiogenic factors and implications for later cardiovascular disease. Circul. 2011; 123 (24): 2856-69.
3. Cassandra, C. N. Dyslipidemia and the risk of pre-eclampsia: genetic causes and related modifiers. PhD Thesis. Thesis, University of IOWA. 2014.
4. Cindrova-Davies, T. Gabor Than Award Lecture 2008: pre-eclampsia–from placental oxidative stress to maternal endothelial dysfunction. Placenta.2009; 30: 55-65.
5. Chaiworapongsa Tinnakorn, Chaemsaithong Piya, Yeo Lami et al. Preeclampsia part 1: current understanding of its pathophysiology. Nat Revi Nephrol. 2014;10 (8): 466-80.
6. Bhadarka, Nagajan; Mukherjee, Tarak Nath. Risk factors of early and late onset preeclampsia in population admitted at Gujurat Adani Institute of medical science, Bhuj, Kutch, Gujurat, India. Int J Curr Res Life Sci. 2016; 5: 569-72.
7. Lisonkova, Sarka; Joseph, K. S. Incidence of preeclampsia: risk factors and outcomes associated with early-versus late-onset disease. Am J Obstet Gynecol. 2013; 209 (6): 544: e1-544. e12.
8. Shu-Han You, Po-Jen Cheng, Ting-Ting Chung, et al. 2018;18: 199. doi.org/10.1186/s12884-018-1845-7
9. Gomathy, E.; Akurati, Lahari; Radhika, Kondareddy. Early-onset and late-onset preeclampsia-maternal and perinatal outcomes in a rural tertiary health center. Int J Reprod Contracept Obstet Gynecol. 2018; 7 (6): 2266-9.
10. LI, Xun, et al. Similarities and differences between the risk factors for gestational hypertension and preeclampsia: A population based cohort study in south China. Pregnancy Hypertension. Int J Women's Cardiovascul Health. 2016; 6 (1): 66-71.
11. Yazdani, Maryam, Aria Shakeri, et al. Prenatal and maternal outcomes in advanced maternal age, a comparative study. Women's Health Bulletin. 2015; 2(2): e23092.
12. Vavina, O. V., Khodzhaeva, Z.S, Vyssokikh, M.Yu, et al. Profound mitochondrial dysfunction leads to early onset preeclampsia. Eur J Obstet Gynecol Reprod Biol. 2016;206: e118.