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EXPRESSION TROPHOBLAST CELL *B-CELL LYMPHOMA (BCL2)* IN EARLY AND LATE-ONSET PREECLAMPSIA

EKSPRESI PROTEIN *B-CELL LYMPHOMA (BCL2)* SEL TROFOBLAS PADA PREEKLAMPSIA AWITAN DINI DAN AWITAN LAMBAT

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ABSTRACT

Background: Anti-apoptotic Bcl-2 has an important role that is involved in the regulation *of apoptosis. Abnormal apoptotic activity in preeclampsia is caused by the dysregulation of these proteins. Trophoblast antiapoptotic and proapoptotic imbalance is thought to have different influences on the process of early-onset preeclampsia and late-onset preeclampsia*

Objective*: to analyze the difference in Bcl-2 expression in early and late-onset preeclampsia.*

Method of study*: This method was conducted using a cross-sectional study in early-onset preeclampsia compared to late-onset preeclampsia in RSUP. DR. Sardjito Yogyakarta was conducted from April 2019 to June 2019 with consecutive sampling methods. Placental tissue samples were obtained from 26 pregnancies with early-onset preeclampsia and 33 pregnancies with late-onset preeclampsia. The placental expression of Bcl-2 has been investigated by immunohistochemical staining and used semi-quantitative HSCORE examination.*

Results*: The T-test study showed there was a significant difference in Bcl-2 expression among early onset preeclampsia (2.31±0.66) and late-onset preeclampsia (2.61±0.43) with p-value 0.047 (p<0.05). Bcl-2*
the expression appears lower in early-onset preeclampsia with a mean value of 0.30 (CI 0.04 – 0.60).

Conclusion*: Thus we can conclude the expression of Bcl-2 is considered lower in early-onset preeclampsia compared to late-onset preeclampsia.*

Keywords*: Bcl-2; Early onset preeclampsia; Late onset preeclampsia ; Trophoblast*

ABSTRAK

Latar belakang: Protein Bcl-2 merupakan anti - apoptosis yang memiliki peranan penting dalam regulasi proses apoptosis. Gangguan regulasi protein Bcl-2 tampak pada kehamilan

dengan preeklamsia. Patomekanisme ketidakseimbangan antiapoptosis dan proapoptosis pada sel trofoblas diduga memiliki pengaruh yang berbeda pada preeklampsia awitan dini dan preeklampsia awitan lambat.
Tujuan: untuk menganalisis perbedaan ekspresi Bcl-2 sel trofoblas plasenta pada preeklampsia awitan dini dan preeklampsia awitan lambat.
Metode penelitian: Penelitian menggunakan studi potong lintang pada pasien preeklampsia awitan dini dibandingkan dengan preeklampsia awitan lambat di RSUP. DR. Sardjito Yogyakarta yang dilakukan pada bulan April 2019 hingga Juni 2019 dengan metode pengumpulan sampel secara *consecutive sampling*. Sampel jaringan plasenta didapatkan dari 26 kehamilan dengan preeklampsia awitan dini dan 33 kehamilan dengan preeklampsia awitan lambat. Ekspresi Bcl-2 plasenta dinilai dengan pewarnaan imunohistokimia dan menggunakan pemeriksaan HSCORE semi-kuantitatif.
Hasil: uji T menunjukkan adanya perbedaan yang signifikan pada ekspresi Bcl-2 antara preeklampsia awitan dini (2,31±0,66) dengan preeklampsia awitan lambat (2,61±0,43) dengan nilai p-value 0,047 ($p<0,05$). Ekspresi Bcl-2 tampak lebih rendah pada preeklampsia awitan dini dengan nilai rata-rata 0,30 (CI 0,04 - 0,60).
Kesimpulan: Ekspresi Bcl-2 pada sel trofoblas plasenta lebih rendah pada preeklampsia awitan dini dibandingkan dengan preeklampsia awitan lambat.

Kata kunci: protein Bcl-2, preeklampsia awitan dini, preeklamsia awitan lambat, trofoblas

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BACKGROUND

Preeclampsia interferes with 7 – 10 % of pregnancy proportions in the United States and records 3,9 % of pregnancy occurrences (Cunningham *et al*.,2014). Preeclampsia is one of the main causes of maternal mortality which is reported in East Java at as high as 31% and in Central Java at 34 % (Dinas Kesehatan Jawa Timur, 2016; Dinas Kesehatan Jawa Tengah, 2016). Up until today, preeclampsia is still described as a "disease of theories" based on multiple theories of etiology and pathogenesis pathway. Trophoblast invasion in spiral artery failure later causes changes in adaptive physiology in pregnancy (Roberts and Escudero, 2012).

Apoptosis plays a major role in normal placenta development, while any disturbances may cause pathological sequences. This mechanism might be involved in trophoblast invasion, spiral artery transformation, trophoblast transformation, and labor process. B-cell lymphoma (Bcl-2) specifically is an important anti-apoptotic protein that acts to prevent the release of cytochrome C from mitochondria and its expression is higher in syncytiotrophoblast compared to cytotrophoblast. This number expressed is believed to

prevent the syncytiotrophoblast after the synced fusion step. Disturbance in these process is account for preeclampsia occurrence and later give distinguish outcome in early and late-onset preeclampsia (Huppertz, 2008 ; Straszewski-Chavez, Abrahams and Mor, 2005). This study aims to assess the Bcl-2 expression difference in early-onset and late-onset preeclampsia.

METHODS

A cross-sectional study was conducted on early-onset and late-onset preeclampsia in RSUP. Dr. Sardjito Yogyakarta. The inclusion criteria are preeclampsia or eclampsia women with 24-40 weeks of gestational age and agreed to be the subject of this study. Exclusion criteria such as chorioamnionitis, chronic renal failure, SLE, diabetes mellitus, and chronic hypertension. The expected number of subjects in this study is 36 in each category.

The study was conducted from April to June 2019 with ethical clearance obtained from the Medical Faculty of Gadjah Mada University. The sample was drawn from the placenta right after the birth of the baby. The sample was then sent to the Histology laboratory Medical Faculty of Gadjah Mada University. Healthy placenta tissue was properly selected and was cut twice, each size 2 x 2 x 1 cm3, and transported in a 100 cc pot containing 80 cc formalin buffer. The fixation process was carried out in the next 24 hours and further put in 70% alcohol for another 24 hours. The paraffin block later took place for IHC coloring and with magnifying effect of 40x, HSCORE can be assessed. The HSCORE formula = Σ Pi. (i+1), Pi is positive cell percentage, i is for color intensity hence measured as 0 = negative, 1= mild positive, 2 = moderate, and 3 = strong.

RESULT

The sample was collected over 3 months period, starting from April until June 2019. There were 59 placenta samples divided into 2 groups, an early-onset preeclampsia group consisting of 26 samples, and late-onset preeclampsia with 33 samples.

Table 1. Subject characteristics in both study groups

Variable	Early onset preeclampsia (n = 26) Mean ± SD	Late-onset preeclampsia (n = 33) Mean ± SD	p-value
Age (years)	32.81 ± 6.6	26.94 ± 6.20	0.00*
Age of gestations (weeks)	33.15 ± 2.79	37.58 ± 1.68	0.00*
Parity	2.23 ± 1.45	1.48 ± 0.71	0.01*
BMI (kg/m^2)	30.41 ± 8.69	28.53 ± 5.69	0.32

**T-test significant p<0.05*

Table 1 shows subject age in the early onset preeclampsia has a higher mean (32.81) compared to the late onset preeclampsia group (26.94), and is significantly different (p<0.05).

The parity variable shows significant differences between both study groups, whose higher parity is in the early onset preeclampsia (p<0.05). There is no significant difference in BMI variables in both study groups. A normality test was carried out and showed that the p-value> 0,05 define that the study data has been normally distributed, furthermore the independent t-test was done in this study.

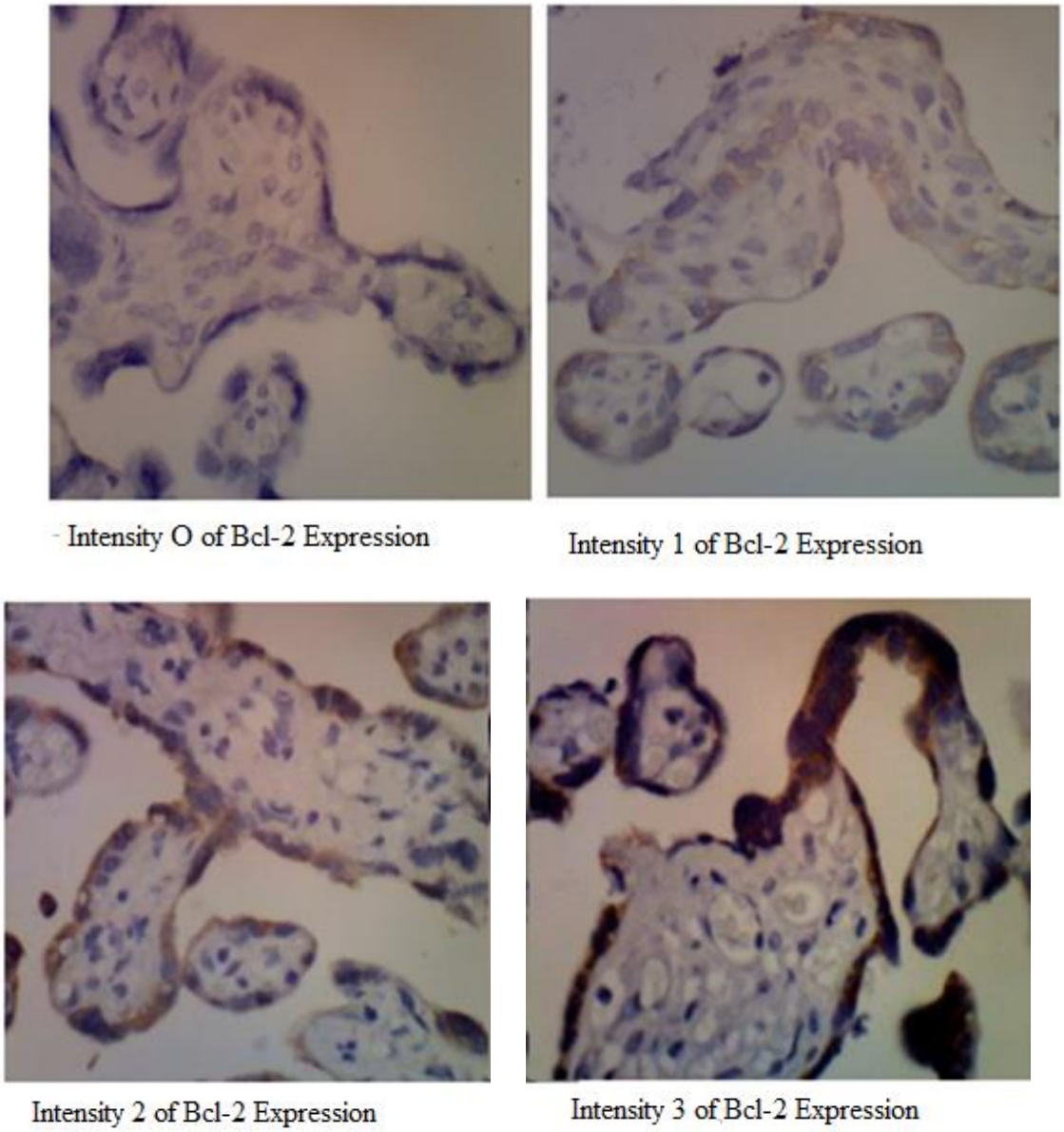

Figure 1. The Intensity expression of Bcl-2

Table 2. T-test study comparison Bcl-2 expression on early onset and late-onset preeclampsia

Variable	N	*Mean*	*SD*	Δ *Mean*	95%*CI*	*P*
Preeclampsia						
Early onset	26	2.31	0.66	0.3064	0.04 – 0.60	0.047*
Late-onset	33	2.61	0.43			

**T-test significant p<0.05*

Table 2 reveals the mean comparison of Bcl-2 expression in both studies using *an independent T-test.* The mean Bcl-2 expression is lower in the early onset preeclampsia

group (2.31±0.66) and statistically significant compared to the later group 0.30 (CI 0.04 – 0.60**)**

DISCUSSION

There is a significant difference in subject characteristics visible in this study, such as the mother's age and parity showed significantly higher in the early-onset preeclampsia group compared to the late-onset preeclampsia group. Meanwhile, the BMI characteristics are not different significantly. Previous studies showed both early-onset and late-onset preeclampsia have the same risk factors as those developing chronic hypertension and solely give rise to 10 times higher early-onset preeclampsia and 5 times higher in late-onset preeclampsia compared to normal pregnancy. A very young age (< 20 years old), nullipara, and previous history of diabetes mellitus have a higher risk of developing late-onset preeclampsia (Lisonkova and Joseph, 2013; Aksornphusitaphong, 2013; Lacobelli , Bonsante and Robillard, 2017). Older age and chronic hypertension have a higher risk for early onset preeclampsia (Shu-Han You *et al*., 2018). Those previous studies correlate well with the result of this study, which showed older age and higher parity will more likely to develop early-onset preeclampsia. Body mass index did not reveal any significant difference in this study. This the result has similarities with previous studies (Lisonkova and Joseph, 2013; Shu-Han You *et al*., 2018) while another study by Valensise *et al* showed that a higher BMI has a higher risk of developing late-onset preeclampsia compared to early-onset preeclampsia (Valensise *et al*., 2008).

The protein expression means the difference in both groups is 0.3 (CI 95 % 0.04 -0.6). Based on those data, we later understand that the Bcl-2 protein expression is lower in early onset compared to late-onset preeclampsia. Previous studies generally investigating preeclampsia showed Bcl-2 expression as an anti-apoptosis agent is lower compared to normal pregnancy (Aban *et al*., 2004; Cali *et al*., 2013; Arianto, Hadiati, and Nurdiati, 2015). Previous studies concerning the early onset and late-onset preeclampsia showed protein expression differences in Bcl-2, EPO, and Ki-67 in syncytiotrophoblast, macrophage in villous stromal and capillary endothelial, which specify the Bcl-2 expression in syncytiotrophoblast and macrophage is lower in late-onset preeclampsia, while higher expressed in endothelial capillary (Medvedev, Syundyukova and Sashenkov, 2016; Vasilii *et al*., 2017). The study by Vavina *et al*.,(2016) showed lower Bcl-2 expression as well in early-onset preeclampsia compared to late-onset preeclampsia (Vaviva *et al*., 2016). These findings are consistent with the result of the previous study which showed lower expression in early-onset preeclampsia (2.31±0.66) compared to the kate onset preeclampsia (2.61± 0.43) with a mean difference of 0.30 (CI 0.04 – 0.60**).**

ROS increase finding in early-onset preeclampsia will trigger apoptogenic factors and further cause oxidative stress in placental mitochondria, later showing high-grade oxidative stress in early-onset preeclampsia (Vaviva *et al*., 2016). This event will activate the apoptosis mechanism through the activation of extracellular regulated kinase (*ERK)*, p38 Mitogen-Activated Protein Kinase(*MAPK*), and protein c-Jun N-terminal kinase (*JNK*) protein (Aouache *et al*., 2018). While in late-onset preeclampsia, the oxidative stress will increase glutathione peroxidase, to harbor cellular function while there is a disruption in organ level. This specific mechanism will not occur in early-onset preeclampsia, hence the decrease of SOD later cause an immune response in severe preeclampsia. These SOD deficits in mitochondria later cause lower concentrations of H2O2 (Holland *et al*., 2018).

Activation of apoptosis p53 signal from mitochondria leads to protein BAX and BAK activity which belong to the proapoptotic family of Bcl-2 which later causes an increase of mitochondrial membrane permeability toward apoptosis inducer agent, cytochrome C

(Straszewski-Chavez, Abrahams and Mor, 2005). In early-onset preeclampsia, there is an increased apoptosis ratio of BAX/Bcl-2, while in late-onset there is a decreased amount of pro-apoptosis BAX and an increase of Bcl-2. These findings showed the occurrence of mitochondrial suppression towards the apoptosis signal thus further causing placental cell survival in late-onset preeclampsia towards a hypoxia state that was absent in early-onset preeclampsia (Holland *et al*., 2018).

The limitation of the study is the data obtained is only known during hospital admission. The antenatal care information is lacking. The delay time during the real onset of preeclampsia and hospital admission might bias the group classification process. Another limitation of this study was the sample size which was only 59 (total) obtained hence the power of the test was as low as 0,62.

CONCLUSION

Bcl-2 expression in early-onset preeclampsia is lower compared to those in late-onset preeclampsia.

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