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Comparison of Bcl-xL protein expression in placental trophoblast cells between pregnancy complicated by severe preeclampsia and normotensive pregnancy

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ABSTRACT

Preeclampsia is one of the main causes of maternal and perinatal mortality and morbidity. The pathogenesis of preeclampsia remains unclear until now. It is believed that regulation of apoptosis in trophoblast cells plays an important role in the pathophysiology of preeclampsia. Failure of spiral arteries remodeling will eventually lead to placental hypoxia lead to excessive trophoblast apoptosis. The molecular mechanism of apoptosis is very complicated involving many signaling molecules included Bcl-2 proteins. The Bcl-2 protein group consists of proapoptosis proteins (Bax) and apoptosis inhibitor proteins (Bcl-2 and Bcl-xL). The aimed of this stuty was to compare the expression of Bcl-xL protein in placental trophoblast cells of pregnancy complicated by severe preeclampsia with that normotensive pregnancy. This study was an observational study with cross sectional design involving 43 pregnancy patients with severe preeclampsia and 38 normotensive pregnancy who treated in Dr. Sardjito General Hospital, Yogyakarta from October 2011 until March 2012. Placenta samples were obtained from all subjects for Bcl-xL protein expression analysis using immunohistochemistry technique. Data were analyzed using independent t-test, chi-square test, and logistic regression. A p value <0.05 was considered significant. Significant difference in Bcl-xL protein expression in trophoblast cells of pregnancy complicated by severe preeclampsia (1.29 ± 0.12) compared to that normotensive pregnancy (1.71 ± 0.14) was reported ($p = 0.00$). In addition, logistic regression test showed that diagnosis of severe preeclampsia had a statistically significant role in Bcl-xL protein expression ($p = 0.000$). In conclusion, the expression of Bcl-xL protein is lower in pregnancy complicated by severe preeclampsia compared to normotensive pregnancy.

ABSTRAK

Preeklamsia merupakan salah satu penyebab utama mortalitas dan morbiditas maternal dan perinatal. Patogenesis preeklamsia masih belum jelas sampai saat ini. Diduga pengaturan apoptosis pada sel trofoblas memegang peranan penting dalam patofisiologi preeklamsia. Kegagalan remodeling arteri spiralis akan menyebabkan hipoksia pada plasenta dan apoptosis trofoblas yang berlebihan. Mekanisme molekuler apoptosis sangat komplek yang melibatkan banyak molekul sinyal termasuk protein Bcl-2. Kelompok protein Bcl-2 terdiri dari protrin proapoptosis (Bax) dan penghambat apoptosis (Bcl-2 dan Bcl-xL). Penelitian ini bertujuan untuk membandingkan ekspresi protein Bcl-xL sel trofoblas plasenta pada kehamilan dengan preeklamsia berat dengan kehamilan nomal. Penelitian

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observasi dengan rancangan potong lintang ini melibatkan 43 pasien wanita hamil dengan preeklamsia berat dan 38 wanita hamil normotensi yang dirawat di RSUP Dr. Sardjito, Yogyakarta antara Oktober 2011 sampai Maret 2012. Sampel plasenta diambil dari semua subjek untuk pemeriksaan ekspresi protein Bcl-xL menggunakan teknik imunohistokimia. Data yang diperoleh dianalisis denga uji t independen, uji chi square dan uji regresi logistic. Nilai $p < 0.05$ digunakan sebagai dasar menyatakan perbedaan nyata. Dijumpai perbedaan nyata ekspresi protein Bcl-xL pada sel trofoblas kehamilan dengan preeklamsia berat (1,29 ± 0,12) dibandingkan dengan kehamilan normotensi (1,71 ± 0,14) ($p = 0,00$). Selain itu, uji regresi logistik menunjukkan diagnosis preeklamsia berat mempengaruhi secara nyata terhadap ekspresi protein Bcl-xL ($p = 0,000$). Dapat disimpulkan bahwa ekspresi protein Bcl-xL lebih rendah pada kehamilan dengan preeklamsia berat dibandingkan dengan kehamilan normotensi.

Keywords: trophoblast - severe preeclampsia - Bcl-xL protein – apoptosis - normotensive

INTRODUCTION

Preeclampsia is one of the main causes of maternal and perinatal mortality and morbidity. The pathogenesis of preeclampsia remains unclear until now. However, it is believed that the failure of spiral arteries remodeling will eventually leads to placental hypoxia. This theory may not be the main cause of preeclampsia, but at least it is involved in the pathogenesis of this disease.[1, 2]

Apoptosis has an important role not only in the development of placenta but also in the pathophysiology of pregnancy complicated by preeclampsia. Apoptosis of trophoblast cells is increasing with gestational age and this increase has been studied as a complication of pregnancy with preeclampsia and intrauterine growth restriction (IUGR). Although this hypothesis is still under study, it is believed that regulation of apoptosis in trophoblast cells plays a key role in the pathophysiology of preeclampsia.[3, 4]

The molecular mechanism of apoptosis in human is very complicated involving many signaling molecules included Bcl-2 proteins. The Bcl-2 protein family consists of proapoptotic protein such as Bak and Bax, and apoptosis inhibitor protein such as Bcl-2 and Bcl-xL. During pregnancy, Bcl-2 is found in placenta since first trimester of pregnancy until third trimester and the concentration is decreasing with gestational age. In pregnancy complicated by preeclampsia, regulators of placental apoptosis are expressed differently. Several recent studies have found that expression of Bcl-2 and Bcl-xL as antiapoptotic molecules in patients with severe preeclampsia and IUGR are lower than patients with normal pregnancy.[3, 4]

The purpose of this study was to compare the expression of Bcl-xL protein in placental trophoblast cells of pregnancy complicated by severe preeclampsia with normotensive pregnancy. This study also aimed to evaluate the effect of maternal age, gestational age, and maternal mean arterial pressure (MAP) in the expression of Bcl-xL protein.

MATERIALS AND METHODS

Subjects

This was an observational study with cross sectional design. The population were patients with severe preeclampsia and normotensive patients who treated in Dr. Sardjito General Hospital, Yogyakarta, Indonesia from October 2011 until March 2012. The inclusion criteria

were patients with severe preeclampsia in 28-40 weeks of gestational age and agreed to be included in the study. The exclusion criteria were presence of comorbid diseases such as chorioamnionitis, chronic hypertension, diabetes, systemic lupus erythematosus, sickle cell disease, thyroid diseases, heart diseases, bronchial asthma, seizure which was caused by other etiologies beside preeclampsia, HIV, and fetus with major congenital disorder. Written inform consent were obtained from each patient after sufficient information was given.

Protocol of study

Samples were taken from the placenta immediately after the baby was born. Samples were then sent to Histology Laboratory, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia. Samples of placental tissue were stained using immunohistochemistry technique to measure the expression of Bcl-xL protein. The expression of Bcl-xL protein was reported to be positive if brown color was found in cytoplasm or cell membrane. The expression of Bcl-xL protein was measured using semiquantitative immunohistochemical scoring system (HSCORE). The formula was HSCORE = $\sum Pi\ (i+1)$, where *Pi* was percentage of cells which are stained positively with Bcl-xL immunostaining and *i* was intensity of staining with different grades (0=negative; 1=weakly positive; 2=moderately positive; 3=strongly positive). The HSCORE measurement was conducted by two observers with concealment of sample's identity. Inter observer agreement was tested using kappa test and the result of kappa value was 0.88 which showed that there was a strong agreement between two observers. Expression of Bcl-xL was observed with microscop using high magnification (400x) in 5 fields of view from each samples. The protocol of the study was approved by the Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Statistical analysis

All numerical data were presented as mean ± standard deviation (SD). Statistical analysis was conducted using independent t test to evaluate the difference in mean between two groups. Bivariate analysis using chi-square test was used to evaluate correlation between two categorical variables. Multivariate analysis using logistic regression was applied to evaluate the relationship between independent variable (preeclamptic vs normotensive group), confounding variables (patient's age, gestational age, and mean arterial pressure), and dependent variable (Bcl-xL protein expression). A p value less than 0.05 was considered statistically significant.

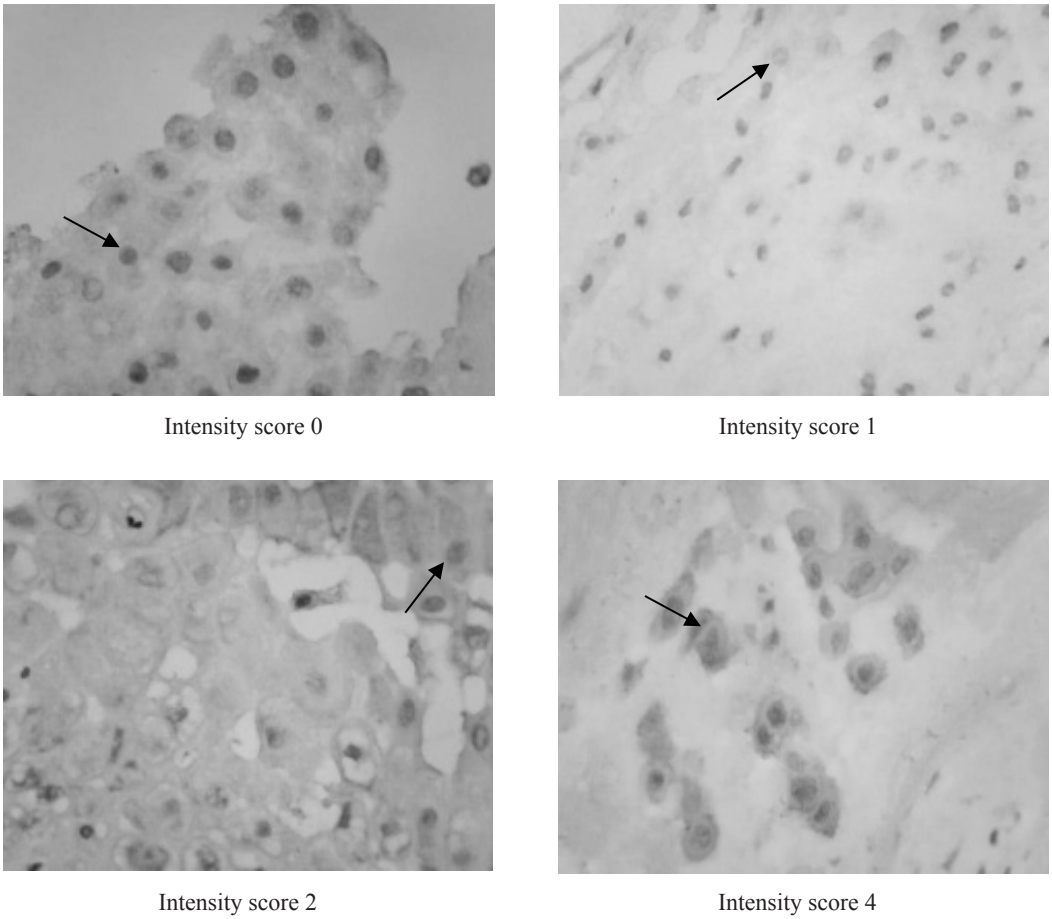
RESULTS

Placenta samples were obtained from 43 patients with pregnancy complicated by severe preeclampsia and 38 patients with normotensive pregnancy. All samples were stained using immunohistochemistry technique to evaluate the expression of Bcl-xL protein Characteristics of subjects of normotensive and severe preeclamptic groups are shown in TABLE 1. No significantly different of patient's age of severe preeclamptic compared to normotensive group was observed ($p>0.05$). Meanwhile, there were significant differences of gestational age and MAP of both groups ($p<0.05$).

TABLE 1. Characteristics of subjects (mean ± SD) of normotensive and severe preeclamptic groups

Variable	Normotensive Group (n=38)	Severe Preeclamptic Group (n=43)	Mean Difference (95% CI)	p
Patient's age (years)	28.42 ± 6.77	28.37 ± 7.08	0.5 (-3.0136 – 3.116)	0.974
Gestational age (weeks)	35.67 ± 3.01	38.39 ± 1.85	-2.72(-3.82 - -1.63)	0.000
Mean arterial pressure (mmHg)	126.29 ± 17.00	88.95 ± 6.44	37.34 (31.74-42.94)	0.000

In this study, the expression of Bcl-xL protein was observed in decidual trophoblast. In accordance with early onset preeclampsia theory which stated that the failure of spiral artery remodelling occured in decidual layer. The change in intensity of brown color in trophoblast cells of decidual layer corresponded to expression of Bcl-xL as shown in FIGURE 1.



Intensity score 0 Intensity score 1

Intensity score 2 Intensity score 4

FIGURE 1. Intensity of color in Bcl-xL protein staining

TABLE 2 shows comparison of Bcl-xL protein expression in placenta of severe preeclamptic and normotensive group. The mean expression of Bcl-xL protein in severe preeclamptic group was significantly lower than that normotensive group (p=0.00).

TABLE 2. The Bcl-xL protein expression (mean ± SD) in placenta of severe preeclamptic and normotensive groups

Variable	Severe Preeclamptic Group (n=43)	Normotensive Group (n=38)	Mean Difference (95% CI)	p
Bcl-xL Expression	1.29 ± 0.12	1.71 ± 0.14	- 0.42 (-0.47 – -0.36)	0.00

Receiver operating characteristic (ROC) analysis was conducted to determine the cutoff point of Bcl-xL protein expression. The analysis found that the area under curve (AUC) was 0.93 or 93% with cutoff point of 1.495 as shown in FIGURE 2.

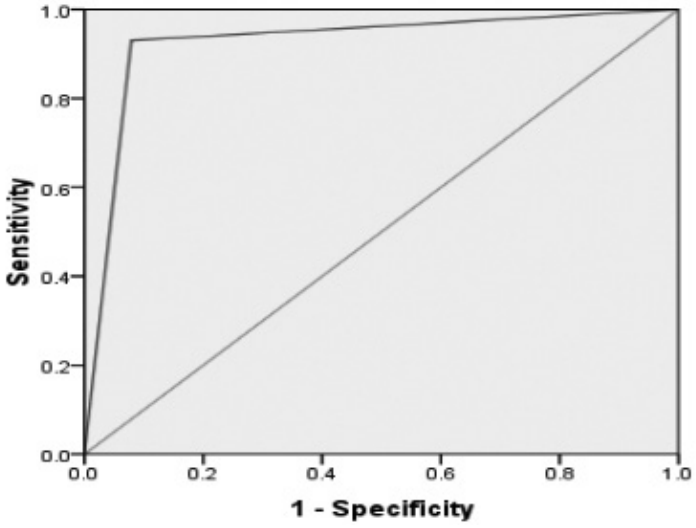


FIGURE 2. ROC curve of Bcl-xL protein expression

TABLE 3 shows bivariate analysis between independent and dependent variable. The pregnant patients with severe preeclampsia had more chances to have expression of Bcl-xL protein valued less than 1.495 compared to that normotensive patients, with relative risk of 11.78 (3.96-35.01) and p=0.000.

TABLE 3. Bivariate analysis between independent and dependent variable

Variable	Bcl-xL expression		%	RR (95% CI)	p
	<1,495	≥1,495			
Severe preeclamptic group	40	3	93.0	11.78 (3.96-35.01)	0.000
Normotensive group	3	35	7.9		

Bivariate analyses between confounding variables and the expression of Bcl-xL protein were shown in TABLE 4. Patient's age did not significantly affect the expression of Bcl-

Xl protein [RR= 0.93 (0.03-31.7); p >0.05]. In contrary, gestational age and MAP affected the expression of Bcl-Xl protein (RR =0.38 (0.25-0.58; p<0.001) and [RR= 2.67 (1.83-3.91); p<0.001], respectively.

TABLE 4. Bivariate analyses between confounding variables and expression of Bcl-xL protein

Variables	Bcl-xL expression		%	RR (95% CI)	p
	<1,495	≥1,495			
Patient's Age					
<20 and >40 years old	7	7	50	0.93	>0.05
20-40 years old	36	31	54	(0.03-31.7)	
Gestational Age					
≥37 weeks	18	35	34	0.38	<0.001
<37 weeks	25	3	89	(0.25-0.58)	
Mean Arterial Pressure (MAP)					
>123 mmHg	24	2	92.3	2.67	<0.001
≤123 mmHg	19	36	34.5	(1.83-3.91)	

Multivariate analysis using logistic regression between independent variable, dependent variable, and confounding variable is shown in TABLE 5. Diagnosis of severe preeclampsia consistently affected the expression of Bcl-xL protein (p=0.000), while gestational age and MAP did not affect the expression of Bcl-xL protein (p>0.05).

TABLE 5. Multivariate analysis using logistic regression between independent variable, dependent variable, and confounding variable

Variables	OR	95% CI	p
Diagnosis of severe preeclampsia	220,036	14.62-66.12	0.000
Patient's age	2.63	0.286-24.260	0.393
Gestational age	1.219	0.101-14.728	0.876
Mean arterial pressure	0.94	0.072-12.301	0.965

DISCUSSION

No significantly different in patient's age between severe preeclamptic group and normotensive group was found in this study. Previous studies reported that women with severe preeclampsia were older than normotensive pregnancy although it was not statistically significant.[5-7] Meanhwile, other studies found that preeclampsia were more common in women with advanced maternal age (≥35 years old) compared to younger women. Advanced maternal age is an independent risk factor for adverse outcomes in first-time mothers with preeclampsia.[8, 9]

Mean gestational age in severe preeclamptic group was significantly lower than normotensive group indicating an inhomogenous distribution in research samples. This result was in accordance with a study by Zhang *et al.*[7] which found that mean gestasional age in severe preeclamptic group was significantly lower than control group. On the contrary, Sharp *et al.*[6] and Allaire *et*

al.[10] found no difference in gestasional age between preeclamptic group and control group.

This study also found that MAP in severe preeclamptic group was higher than in normotensive group. This could be understood clearly because in preeclampsia there will be an increase in blood pressure.

This study found that expression of Bcl-xL protein as antiapoptotic molecule in patients with preeclampsia was lower than normotensive patients. This was in accordance with a study by Shu *et al.*[11] which found that Bcl-xL expression was down-regulated in preterm preeclampsia, but not in term preeclampsia and controls. Allaire *et al.*[10] concluded that the presence of apoptotic marker could be used as a sign of intrauterine hypoxia. Hung *et al.*[12] found that hypoxia and prolonged hypoxia-reoxigenation seemed to cause more reduction in the levels of Bcl-xL, although the difference was not statistically significant. Meanwhile, a study by Zhang *et al.*[7] found that Bcl-xL mRNA expression levels was unchanged in severe preeclamptic placentas when compared to control. In contrast, Whitehead *et al.*[13] found significantly increased placental RNA expression of Bcl-xL in early onset FGR (fetal growth restriction), PE complicated by FGR, and PE without FGR compared with preterm controls. This perhaps reflects a disordered regulation of apoptosis in placental dysfunction that is as yet not clearly understood.

No correlation between maternal age and expression of Bcl-xL protein as antiapoptotic molecule was observed in this stuudy. In contrast, Kavathia *et al.*[14] reported that there was a positive linear correlation between apoptosis and person's age. The discrepancy could be caused by group arrangement in this study which was based on risk factor of preeclampsia, where patient with age<20 years old and >40 years old were considered in high risk, and patient between 20-40 years old were in low risk.

This study also found that there was a correlation between gestational age and expression of Bcl-xL protein. In normal condition, apoptotic activity is increasing with gestational age. Previous study by Smith *et al.*[15] which compared apoptosis in normotensive pregnant women from first trimester and third trimester found that there was an increase in apoptosis index in third trimester. In normal condition, antiapoptotic expression is decreasing with gestational age as shown in a study by Kim *et al.*[16] which found that that there was a decrease in expression of Bcl-2 protein, an antiapoptic molecule, in third trimester.

Abnormality in apoptosis stimulation in patients with essential hypertension showed that antiapoptotic factors concentration in those patients were decreased and it could be caused by ischemia.[17] This was in accordance with this study where bivariate analysis between MAP and expression of Bcl-xL found that patients with MAP≥123 mmHg had 2.67 increases in probability to have expression of Bcl-xL <1.50 compared to patients with MAP<123 mmHg.

CONCLUSIONS

In conclusion, this study found that expression of Bcl-xL protein is lower in pregnancy complicated by severe preeclampsia compared to normotensive pregnancy. In addition, diagnosis of severe preeclampsia consistently affect in the expression of Bcl-xL protein.

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