



Effect of Curcumin in Decreasing MDA Level in Preeclampsia-Induced Human Umbilical Vein Endothelial Cell (HUVEC)

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Abstract : Preeclampsia is multisystem disease occurred in 3-8% pregnancy, indicated by hypertension and proteinuria after 20 weeks of gestational age. In preeclamptic patients, pro-oxidant decrease enzymatically in cell, followed by increase in lipid peroxida due to free radicals *malondialdehyde* (MDA). Elevated free radicals in preeclampsia is associated with reduced cellular antioxidants. Curcumin has been known to posses many biological activities, such as antiinflammation and antioxidants. We evaluated effects of curcumin on MDA level in preeclampsia-induced HUVEC cell line. In the present study, we observed the effects of curcumin on MDA level in preeclampsia-induced HUVEC cell line. MDA level was measured with *Thiobarbituric Acid-reactive Substances* (TBARS). The result of the present study showed curcumin decreased MDA level in preeclampsia-induced cell.

Key words : curcumin, MDA, preeclampsia.

Introduction

Preeclampsia is multisystem disease occurred in 3-8% pregnancy, indicated by hypertension and proteinuria after 20 weeks of gestational age.^{1,2} Preeclampsia causes both maternal and perinatal morbidity and mortality worldwide.¹ Clinical manifestation of preeclampsia consists of hyperkoagulopati, hemolysis elevated liver enzyme and low platelets (HELLP), periportal hemorrhage in liver, ischemic lesion, fibrin cumualtion, subcapsular hemorrhage and intrahepatic hematoma or even hepatic rupture, acute renal failure and segmental glomeruloskeloris segmental.¹⁻³ Moreover, preeclampsia causes cramp (eclampsia), headache, blurry vision, scotoma or even blidness, cerebral hemorrhage, brain damage due to ischemia or microinfark and fibrinoid necrosis in brain.^{2,3}

Pathogenesis of preeclampsia remains unclear. Placenta is believed to play the key role.⁵ Placenta is a source and central of any mediators in preeclampsia pathogenesis.^{6,7} Moreover, placenta is also responsible in preeclampsia development, in which there is disturbance in placentation, poor invasion, and abnormal angiogenesis which is the main pathological manifestation.⁷ These events are results from oxidative stress found in placenta in preeclampsia. Studies show that soluble *vascular endothelial growth factor receptor-1* (VEGFR-1) or known as *soluble fms-like tyrosine kinase-1* (sFlt-1) and *soluble endoglin* (sEng), are mediator mechanism between ischemic placenta and oxidative stress with failure in local angiogenesis^{8,9}, that leads to systemic vascular dysfunction.¹⁰ Oxidative stress can also stimulate release of cytokines, antiangiogenics, microparticles and other essential molecules in preeclampsia.¹¹ Antiangiogenic plays role in early development of placental vascular and during trophoblast invasion, and hypoxia is the major regulator on its expression.¹⁰ Previous studies show that oxidative stress induced sflt-1 concentration.¹¹ Elevated sflt-1 caused reduction in placental growth factor (PlGF) and vascular endothelial growth (VEG) signaling.

Oxidative stress is caused by imbalance of pro-oxidant and antioxidant. In preeclamptic patients, pro-oxidant decrease enzymatically in cell, followed by increase in lipid peroxida due to free radicals *malondialdehyde* (MDA). Furthermore, increase lipid peroxida leads to disturbance in cell integrity, uncontrolled lipid peroxida both in cell and tissues that promotes lysis of cell membrane and edhoteles, vascular reactivation, and increase vascular permeability due to activation of *neutrophil esterase*. *Neutrophil esterase* can be used as marker in endothelial dysfunction on molecular level. In preeclampsia, MDA is present in plasma, small blood vessels, and desidua basalis.^{12,13}

Elevated free radicals in preeclampsia is associated with reduced cellular antioxidants. Curcumin is a compound found in *Curcuma longa*.¹⁴ Curcumin has been known to posses many biological activities, such as antiinflammation and antioxidants.¹⁵ Thus, curcumin is considered as a potent approach in treating oxidative stress and inflammation-related diseases.^{15, 16} Hansson, Chen¹⁷ proposed that curcumin can be utilized as alternative therapy for preeclampsia due to its ability in inhibiting regulator protein, as well as NFκB. Recent study found that curcumin reduced MDA level, as well as NFκB expression in preeclamptic rats.¹⁸ In the present study, we observed the effects of curcumin on MDA level in preeclampsia-induced HUVEC cell line.

Materials and Method

Samples used were women at 32-42 weeks of gestational age which were diagnosed preeclampsia and normal pregnancy at the same gestational age from Dr. Hasan Sadikin General Hospital, Indonesia. Research subjects has fulfilled inclusion and exclusion criteria.

Cell culture

HUVEC cell line was grown in *tissue culture flask* (25 cm²) containing RPMI 1640 supplemented with 10% (v/v) FBS *qualified (fetal bovine serum)*, antibiotic-anti-micotic 1% Penicillin G-Streptomycin *Solution Stabilised* dan (1% Fungizone Amphotericin B) and 1% gentamisin, then incubated at 37°C atmosphere 5% (v/v) CO₂. Medium was replaced 2-3 times a day. Cell were the checked every 7 days or untill 90%. *confluent*¹³¹ Cells viability was measured with *trypan blue* on *haemocytometer* under light microscope with 400x magnification.¹⁹⁻²¹

Measurement of MDA level

Cells of 6x10⁵ cell/ml induced with both normal and preeclampsia serum, were placed into 96-well plate, then incubated at 37°C 5% CO₂ (v/v). Furthermore, each wells were washed 3-4 times with PBS 37°C. Various concentrations of curcumin (0; 0,977; 1,953; 3,906; 7,813; 15,625; 31,25; 62,5; 125; 250 µg/ml) were then distributed to each well, incubated for 24 and 48 hours 37°C 5% CO₂ (v/v). Each well was washed with PBS pH 7,4 once for 5 minute, and then centrifuged for 20 minute at 3.000 rpm. Supernatants were carried for measurement of MDA level with *Thiobarbituric Acid-reactive Substances* (TBARS). Cells were given solution containing 15% w/v trichloroacetic acid, 0,375 w/v thiobarbituric acid, 0.25% hydrichloric acid and 0.2% triton X. Samples were then suspended with heating at 100°C for 15 minutes, then centrifuged at 4500 rpm for 10 min. Absorbance was read at 532 nm wavelength.^{20,21}

Data analysis

Data was analyzed with T-test if normally distributed, and Mann Whitney test if not normally distributed. Data was quantitatively analyzed with ANOVA DMRT (Duncans’s Multiple Range Test) to determine the significance among variables in each treatment SPSS 14.

Results and Discussion

MDA levels

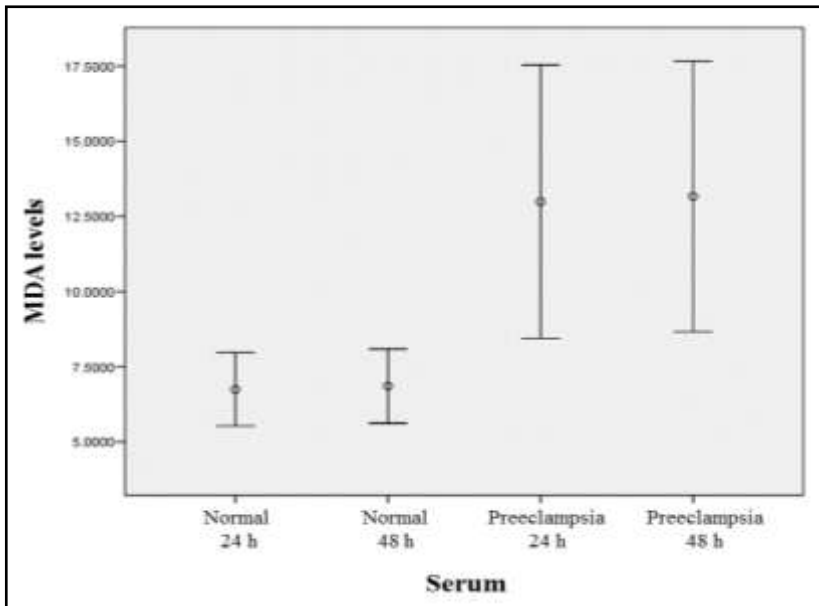


Figure 1. Effects of curcumin in various concentrations and incubation time on level of MDA

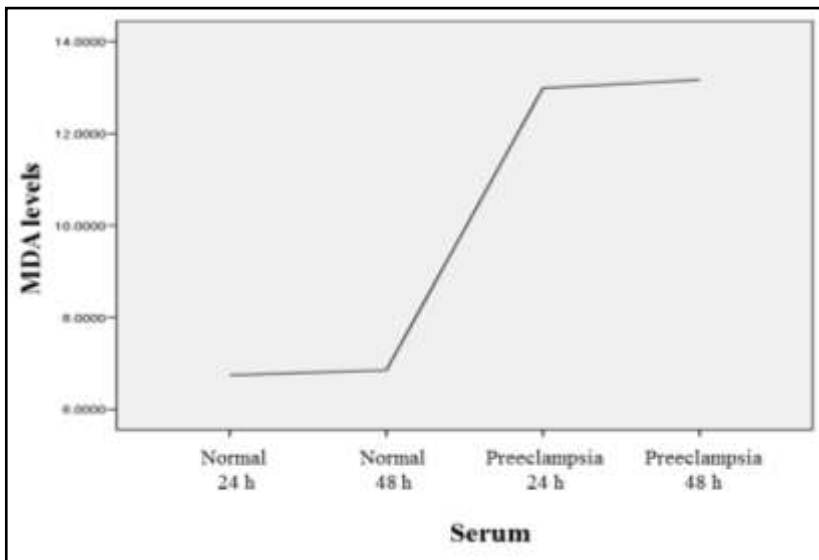


Figure 2. Ratio of MDA levels in preeclampsia-induced HUVEC based on incubation times and curcumin concentration

As shown in Figure 1, MDA level and treatment of curcumin in control and cell, was the highest among treatments. Treatment of curcumin showed effect both in normal and preeclampsia serum after 24 h and 48 h incubation.

Variables tested in this study were normally distributed both in normal and preeclampsia serum treated with curcumin in various concentrations incubated for 24 h and 48 h (data are not shown). Effects of curcumin in various concentration, incubation time, and serums on level of MDA are presented in Figure 2. As shown in Figure 2, level of MDA decreased as in accordance with longer incubation time and increased curcumin concentration. Curcumin decreased level of MDA ($p < 0,001$). Level of MDA in preeclampsia-induced HUVEC decreased from 18,703 μM to 8,577 μM after treated with curcumin of 62,5 $\mu\text{g/ml}$ incubated for 24 h, and higher after 48 h from 18,872 μM to 8,667 μM .

Discussion

Oxidative stress is caused by imbalance of pro-oxidant and antioxidant. In preeclamptic patients, pro-oxidant decrease enzymatically in cell, followed by increase in lipid peroxida due to free radicals *malondialdehyde* (MDA). Furthermore, increase lipid peroxida leads to disturbance in cell integrity, uncontrolled lipid peroxida both in cell and tissues that promotes lysis of cell membrane and edhotels, vascular reactivation, and increase vascular permeability due to activation of *neutrophil estalase*. *Neutrophil estalase* can be used as marker in endothelial dysfunction on molecular level. In preeclampsia, MDA is present in plasma, small blood vessels, and desidua basalis.^{12, 13}

Elevated free radicals in preeclampsia is associated with reduced cellular antioxidants. In the present study, curcumin decreased MDA level in preeclampsia-induced cell. This result is supported by previous study that curcumin significantly attenuated MDA level and recovered the GSH and SOD levels.²² Curcumin is known to protect biomembranes against peroxidative damage. Peroxidation of lipids is known to be a free-radical-mediated chain reaction, leading to the damage of the cell membranes, and the inhibition of peroxidation by curcumin is mainly attributed to the scavenging of the reactive free radicals involved in the peroxidation. Most of the antioxidants have either a phenolic functional group or a -diketone group. Curcumin is an unique antioxidant, which contains a variety of functional groups, including the B-diketo group, carbon-carbon double bonds, and phenyl rings containing varying amounts of hydroxyl and methoxy substituents.²³

Curcumin has been studied recently in treatment of preeclampsia. Curcumin treatment in various doses could decrease significantly pro-inflammatory cytokines levels in preeclamptic plasma-induced. After curcumin treatment, there was decreased level of nuclear NF-kB p50 and increased level of PPAR- γ significantly.²⁴ In other study, LPS-curcumin-treated group had decreased blood pressure and urinary protein level, which was comparable to control group, as well as improved trophoblast invasion and spiral artery remodeling induced by LPS. Increased TLR4, NF-kB and IL-6, MCP-1 protein expressions in LPS-treated group were significantly decreased after curcumin administration.²⁵

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