Effect of Curcumin on sFlt-1 and PlGF Concentration in Preeclampsia Induced HUVEC Cell Line

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ABSTRACT
Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria. Elevated soluble fms-like tyrosine kinase 1 (sFlt-1) and reduced placental growth factor (PlGF) levels are documented in preeclampsia patients. Oxidative stress is known to play a role in the etiopathogenesis of preeclampsia. Hence, antioxidants as physiological protective agents can be used in the treatment preeclampsia. In this study, we assessed the antioxidant properties of curcumin in HUVEC cell line cultured in sera collected from normal and preeclamptic pregnant women. The levels of sFlt-1 and PlGF were measured with ELISA. We found that sFlt-1 level and sFlt-1/PlGF ratio decreased significantly (p<0.05) while PlGF level increased significantly (p<0.05) after the curcumin administration with concentrations ranging from 0.977–250 µg/mL. The levels of sFlt-1, PlGF, and sFlt-1/PlGF ratio in preeclampsia-induced cell were equal to those in normal cell after the addition of 7.813 µg/mL curcumin for 24 h incubation. Similar results were obtained for samples with 48 h incubation, in exception with PlGF level in preeclampsia-induced cell that required 31.250 µg/mL curcumin to be equal to that in normal cell line. In conclusion, curcumin to be used in treating preeclampsia, by restoring the balance between proangiogenic factor (PlGF) and antiangiogenic factor (sFlt-1). However, further studies are warranted to elucidate this potency.

INTRODUCTION
Preeclampsia is a multisystem disorder characterized by hypertension and proteinuria after 20 weeks of gestation, and affects 2 to 5% of pregnancies worldwide.1-3 Preeclampsia is associated with high risks of iatrogenic preterm delivery, intrauterine growth restriction, placental abruption, and perinatal mortality, along with maternal morbidity and mortality.4,5 The underlying mechanism of preeclampsia remains unclear; however, it is suggested to be due to placental malperfusion from the abnormal remodeling of maternal spiral arteries.6-7 Elevated circulating maternal serum levels of soluble fms-like tyrosine kinase 1 (sFlt-1), and reduced placental growth factor (PlGF) levels, are documented in preeclampsia onset.8,9 sFlt-1, an antagonist of PlGF and vascular endothelial growth factor causes vasoconstriction and endothelial damage that may lead to fetal growth restriction and preeclampsia.10-12 The ratio of sFlt-1 and PlGF is associated with an increased risk of preeclampsia which has been approved as a better predictor of risk than either biomarker alone.9,13-16 In preeclampsia, reduced perfusion due to aberrant placentalization and shallow trophoblast invasion promotes placental oxidative stress,17 leading to intravascular inflammatory response and endothelial dysfunction. Many investigations have evaluated antioxidant capacity in the maternal circulation. Although there are mixed results, antioxidant capacity is suggested to decrease in the maternal circulation.18 Hence, the treatment of antioxidants can be used in preeclampsia. Vitamins C and E, nacetylcyysteine, L-arginine, and resveratrol had been used in treatment trial studies, but their effectiveness is conflicting in various studies.19 Exploration of other alternative antioxidants should be considered, especially that of natural products.

Recent studies have been focusing on natural antioxidants from plants due to the presence of phenolic constituents in which they are biodegradable and non-toxic products.20,21 Curcumin (diferuloylmethane) is a naturally occurring yellow pigment isolated from ground rhizomes of the plant Curcuma longa L. (Zingiberaceae). Although molecular mechanisms of action of curcumin are not fully understood, it has been reported to exhibit potent anti-inflammatory, anti-tumor, and hypolipidemic properties.22,23 In addition, the potential of curcumin in treating preeclampsia have been investigated with different biomarkers.24,25 In this study, we evaluated the effect of curcumin on sFlt-1 and PlGF levels in preeclampsia-induced HUVEC cell line.

MATERIALS AND METHOD
Study design
This in vitro study adopted a post-test only control group design. Human Umbilical Vein Endothelial Cell (HUVEC) ATCC CRL 1730 (purchased from American Type Collection Culture) was used and induced with normal and preeclamptic sera that were collected from the normal pregnant women and preeclamptic pregnancy, respectively. Curcumin, purchased from Nanjing Zelang Medical Technology Cina, with a variety of concentrations, was administered to each serum group to investigate its
The analysis of sFlt-1 and PlGF levels, from 2.310 to 5.450 pg/mL, contributed to a significant increase (p<0.05) in the ratio of sFlt-1/PlGF. There was a significantly lower level of sFlt-1 in preeclampsia compared to normal cell line.

Ethical clearance
The study was conducted after the approval from Ethical Review Boards of Health Research, Faculty of Medicine and Dr. Hasan Sadikin Hospital, Bandung. All research subjects were voluntarily required to sign informed consent prior to the study.

RESULTS
Effects of curcumin on sFlt-1
Initial sFlt-1 level in preeclampsia-induced HUVEC cell lines was found to be significantly higher (p<0.001) than in normal cell. sFlt-1 level in preeclampsia-induced cells fell significantly (p<0.05), from 39.545 to 31.135 pg/mL, after incubated for 24 h with 7.813 µg/mL curcumin (Figure 1a). At the same curcumin concentration, sFlt-1 level was reduced surpassing that in the untreated normal cell (31.594 pg/mL). Curcumin suppressed sFlt-1 in a concentration-dependent manner, in which sFlt-1 level was reduced by increasing curcumin concentration. A similar trend was maintained after the incubation of 48 h (Figure 1b).

Figure 1. Effect of different concentrations of curcumin on sFlt-1 in normal and preeclampsia-induced HUVEC cell line with 24 h (a) and 48 h (b) incubation times.

Effects of curcumin on PlGF
There was a significantly lower initial level of PlGF (p<0.001) in preeclampsia induced HUVEC cell line than in normal cell. The addition of curcumin, as low as 1.953 µg/mL, contributed to a significant increase (p<0.05) in PlGF level, from 2.310 to 5.450 pg/mL (Figure 2a). PlGF level in the preeclampsia induced HUVEC cell line (5.847 pg/mL) was close to that in the untreated normal cell line (5.806 pg/mL) when exposed with 7.8125 µg/mL curcumin and incubated for 24 h. However, for samples with 48 h incubation, higher concentration of curcumin (31.250 µg/mL) was required to reach equal PlGF levels between the preeclampsia-induced cell (6.323 pg/mL) and untreated normal cell (6.3145 pg/mL) (Figure 2b).
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Figure 2. Effect of different concentrations of curcumin on PlGF in normal and preeclampsia induced HUVEC cell line with 24 h (a) and 48 h (b) incubation times.

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Ratio of sFlt-1/PlGF
The decreasing trend of sFlt-1/PlGF ratio in response to the increasing curcumin concentration was observed in normal and preeclampsia-induced HUVEC cell lines after 24 h incubation (Figure 3a). The similar trend was also observed in the samples with 48 h incubation time (Figure 3b). Preeclamptic serum-induced HUVEC exhibited higher sFlt-1 and lower PlGF compared to those in normal cells. In samples incubated for 24 h, a significant decrease (p<0.05) in the sFlt-1/PlGF ratio was observed in preeclampsia-induced cell, from 17.151 to 6.466 µg/mL after curcumin treatment with 1.953 µg/mL concentration. Similarly, the significant decrease (p<0.05) was observed at the same curcumin concentration in the 48 h-incubated preeclamptic cells: from 16.758 to 6.437 µg/mL. The ratio decreased surpassing that in the untreated normal cell after the addition of 7.813 µg/mL curcumin and incubation for 24 or 48 h.

Figure 3. Effect of different concentrations of curcumin on sFlt-1/PlGF in normal and preeclampsia-induced HUVEC cell lines with 24 h (a) and 48 h (b) incubation times.

DISCUSSION
The levels of sFlt-1 continue to elevate as placental hypoxia advances, and this is associated with clinical manifestations of preeclampsia, which are elevated blood pressure and proteinuria.30 PlGF continues to reduce in severe preeclampsia which is negatively correlated with sFlt-1. Elevation of circulating sFlt-1 concentration would reduce PlGF concentration, which further results in endothelial dysfunction. This condition occurs a few weeks prior to preeclampsia, therefore can be used as a diagnostic tool of preeclampsia.31-34 Theoretically, when elevated sFlt-1 is found in a woman without clinical manifestation of preeclampsia, the woman will have preeclampsia in the next few weeks.35

Referring to a recent study, sFlt-1 is responsible for oxidative stress, which generates activation of apoptosis in trophoblasts.35 Antioxidant capacity has also been observed to decrease in maternal circulation. Treatment with antioxidants is expected to reduce oxidative stress and inhibit the release of proinflammatory mediators. In the present study, we evaluated the effect of curcumin (diferuloylmethane), which is a compound found in ground rhizomes of Curcuma longa L. This compound has been reported to possess antioxidant activities.22,23

A report suggested that mitochondria dysfunction leading to sFlt-1 production, as the result of reactive oxygen species (ROS) release, can be treated with antioxidants.26
In vitro and in vivo study revealed the effectiveness of mitochondrial-targeting antioxidant (resveratrol) in attenuating sFlt-1 production. The effect of curcumin in suppressing mitochondrial ROS has been reported by some studies. It is in line with our study, where the antioxidant properties of curcumin inhibit the release of sFlt-1 in preeclampsia-induced HUVEC cell line. The trends of the sFlt-1 reduction in HUVEC cells incubated with different times (24 and 48 h) are similar, indicating the antioxidant activities can be maintained after 48 h in the HUVEC cell line. There are previous studies that show the effect of curcumin in preeclampsia. Curcumin decreased the levels of pro-inflammatory cytokines such as IL-1α, IL-6, and TNFα in monocytes culture exposed to plasma preeclamptic by affecting transcription factors of NF-κB and PPAR-γ. Curcumin also improved the preeclampsia-like phenotype in rat models by reducing abnormal inflammation related to TLR4 signaling pathway. Curcumin inhibited the expression of pro-inflammatory factors and macrophage infiltration in the placenta and ameliorated LPS-induced adverse pregnancy outcomes in mice by inhibiting inflammation via upregulation of phosphorylated Akt. We also observed the increase in PI GF level after the curcumin exposure in various concentrations. The sFlt-1 can bind PI GF, which means a reduction of free PI GF concentration, resulting in endothelial damage and microvascular relaxation blockage. Hence, suppression of sFlt-1 by curcumin, in our study, automatically increased the level of PI GF. Nonetheless, other possibilities of endogenous PI GF enhancement by curcumin cannot be ignored. It is corroborated by the different levels of curcumin required to bring the PI GF to have a comparable level with the untreated normal cell line between that incubated for 24 h and 48 h. In samples incubated for 48 h, higher curcumin concentration is required, indicating that reduction of sFlt-1 is not the only explanation as to why the PI GF increased after the curcumin treatment. A study reported the induction of pro-angiogenic agents, VEGF, using curcumin therapy through an unknown mechanism. Treatment using curcumin, therefore, can be potentially used to bring the balance between the pro-and antiangiogenic factors (in this case are PI GF and sFlt-1, respectively). The ratio of sFlt-1 and PI GF obtained after the curcumin exposure on preeclampsia-induced HUVEC cell line may portray such potential, where it is found lower than that in normal vehicle control at 7.813 μg/mL curcumin concentration.

CONCLUSION
Curcumin significantly decreased sFlt-1 and increased PI GF in preeclampsia-induced HUVEC cell line to the levels that are similar to the normal serum. These findings suggest the potential of curcumin to be used in treating preeclampsia, by restoring the balance between proangiogenic factor (PI GF) and antiangiogenic factor (sFlt-1). Further studies including in vivo studies and clinical trials of curcumin are encouraged.

ACKNOWLEDGMENT
We would like thanks HT Editorial Services for the assistance during the manuscript preparation.

REFERENCES
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