CORRELATION OF (PRO)RENIN RECEPTOR AND VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION LEVEL IN THIRD TRIMESTER OF PREECLAMPSIA PLACENTAS

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ABSTRACT

Objective: This cross-sectional study was designed to correlate the expression of mRNA and protein of (pro)renin receptor ([P]RR) and vascular endothelial growth factor (VEGF) gene in preeclampsia placentas.

Methods: We used 34 placenta tissues of normal pregnancy and 34 preeclampsia placenta tissues. Real-time polymerase chain reaction was used to measure relative expression of VEGF and ([P]RR) mRNAs, and protein concentration was measured using Sandwich ELISA technique.

Results: VEGF mRNA relative expression of preeclampsia placenta was 2.83 times higher than the normal placenta (p=0.02). ([P]RR) mRNA relative expression on preeclampsia placenta was 1.7 times higher than the normal one (p=0.039). Expression of protein ([P]RR) and VEGF was lower on preeclampsia shown by p<0.05, R=0.441. From this research, it was considered that low protein of ([P]RR) and VEGF contributes to preeclampsia.

Conclusion: There was a correlation between ([P]RR) and VEGF. It considers that there is any involvement of ([P]RR) on angiogenesis through VEGF expression in preeclampsia placenta.

Keywords: Preeclampsia, Placenta, Vascular endothelial growth factor, (Pro) renin receptor/([P]RR).

INTRODUCTION

One of the major causes of maternal deaths is hypertension during pregnancy. Based on population census data in 2010, 3.2% of maternal mortality was caused by hypertension during pregnancy [1]. One of the hypertension complications during pregnancy is preeclampsia. The incidence rate of preeclampsia in developed countries is range from 2% to 8% [2]. A research conducted by Cho et al showed that the incidence rate of preeclampsia in South Korea is 3.1% [3]. In Indonesia, a research conducted by Warouw and team in RSUP Dr. R. D Kandau Manado showed that the incidence rate of preeclampsia was 6%; meanwhile, a research conducted by Djannah in RSU PKU Muhammadiyah Yogyakarta concludes that the incidence rate of preeclampsia was 16.1% [4,5].

Clinically, preeclampsia is signed by a rise in blood pressure and proteinuria occurs after 20 weeks of pregnancy [5]. Genetically, in Indian woman, preeclampsia is associated with gene polymorphism, and there was significant association between vascular endothelial growth factor (VEGF-C) 405G and VEGF-C [6]. The exact cause of preeclampsia is not known yet until now but is likely to involve several factors such as antiphospholipid antibody syndrome, kidney disease, diabetes mellitus, obesity, systemic erythematous, and nulliparity [7]. The imbalance of angiogenesis factor such as VEGF and renin-angiotensin system (RAS), and antiangiogenesis factor like sFlt-1 is expected as the cause of placenta failure, angiogenesis, and vasculogenesis [8,9]. In normal pregnancy, there is a correlation expression of (pro)renin receptor ([P]RR) with VEGF mRNA [10]. A research conducted by Kanda et al on primary human retinal microvascular endothelial cells (HRMECs) concludes that there is a relation between the activity of ([P]RR) and angiogenesis on retinopathy diabetic [11]. The raise of prorenin concentration will increase the level of VEGF mRNA. That is, why is believed that there is an involvement of ([P]RR) in VEGF regulation mechanism on pathogenesis preeclampsia so that this research is conducted to analyze the expression relation between ([P]RR) and VEGF on placenta of the third-semester pregnant woman with preeclampsia.

METHODS

This research was an observational study with control–case study design. It was conducted at the Laboratory of Molecular Biology for Oxidative Stress, Department of Biochemistry and Molecular Biology, Faculty of Medicine, Universitas Indonesia. It was conducted on January–April 2017. Samples used in this study were collected from the placenta tissues of woman with the third-trimester pregnancy divided into two groups: Normal and preeclampsia (biological material stored). There were 34 tissue’s samples of normal pregnancy and 34 of preeclampsia pregnancy. This research has already approved by Health Studies, Faculty of Medicine Ethics Committee, Universitas Indonesia, number 104/UN2.F1/ETIK/2017.

The total RNA isolation process uses total RNA mini kit (Geneaid). The concentration and purity of RNA were measured using Varioskan. The average concentration of RNA total samples was 175.88 (14.025–588.7) µg/mL, while the average purity index was 1.874 (1.6–2.1).

The analysis of relative expression of ([P]RR) and VEGF mRNA was conducted with reverse transcriptase-PCR technique, using Sensifast™ SYBR No-ROX one-step kit real-time polymermese chain reaction (qRT-PCR) kit as same as Dongare et al [12]. For analysis of ([P]RR) gene, the primer used was forward primer 5’-GAT GGT GAA GGG AGT GAA CAA-3’ and reverse primer 5’-TGG AAT TTG CAA CAC TGT CAA G-3’. For analysis of VEGF gene, the primer used was forward primer 5’-CTG AGG TCT GAA GGG AGT GAA CAA-3’ and reverse primer 5’-CTTTG TCT GCA TTC AC-3’. For reference gene, this research used 18s rRNA gene with primer: Forward...
VEGF mRNA expression increased 2.83 times higher than normal placenta. On placenta tissue with preeclampsia complication, expression of relative mRNA VEGF between preeclampsia placenta and normal placenta. Relative expression of mRNA VEGF was 2.83 times higher than normal samples (p=0.02).

Based on the analysis result, it was found out that mRNA (P)RR expression on preeclampsia placenta was 1.7 times higher than normal placenta. Based on Mann–Whitney test, there was a significant difference of median level of two groups at mRNA (P)RR.

In preeclampsia, the relative expression of mRNA VEGF was 2.83 times higher than the normal samples (Fig 2).

The protein level of VEGF and (P)RR
Based on the result of protein analysis by applying Sandwich ELISA technique, we found out that there was a substantial difference of VEGF protein average between normal group and preeclampsia group (p=0.0001) (Fig. 3). The VEGF protein expression on preeclampsia group was lower than the normal group. The same case also occurred with (P)RR protein concentration. On preeclampsia group, the (P)RR protein concentration average was lower than normal group (p=0.0094) (Fig. 4).

Result data of VEGF protein (Fig. 3) showed that there was a significant difference between preeclampsia and normal placenta, p=0.0001. VEGF protein expression in preeclampsia placentas was lower than in normal placentas.
It showed that VEGF played an important role in vasculogenesis and angiogenesis process. Based on the analysis of VEGF mRNA expression in this research, it was concluded that there were substantial differences of VEGF mRNA expression between preeclampsia and normal group. The VEGF mRNA expression on preeclampsia group increased 2.83 times higher than normal group. The same conclusion was also stated by Chung et al. that there was an increase of VEGF mRNA expression 2.8 times higher than preeclampsia [17]. Escudero et al. also concluded that there was an increase of VEGF mRNA expression on women with preeclampsia syndrome in chilli compared to the normal group [18]. On the other hand, Andraweera et al. who conducted research about group pregnant women in Adelaide Australia concluded differently that the expression of VEGF mRNA of placenta on preeclampsia was lower than the normal group [19].

In this research, protein concentration of VEGF placenta on preeclampsia group was lower than the normal one. The low concentration of VEGF protein on preeclampsia was expected related to the failure of angiogenesis. A research conducted by Gannou et al., about Arabic Tunisian women, found that VEGF plasma concentration on preeclampsia was lower than normal pregnancy [20]. Livingston et al. who conducted a research of pregnant women in Ohio concluded that there was restriction of VEGF serum concentration on serious preeclampsia [21]. Maynard et al., in his research, stated that free VEGF on serum decreased on preeclampsia pregnancy. The increase of sFlt-1 that was alternatively spliced from Flt-1 caused the VEGF bound to Flt-1 decreases and ultimately might cause problems in signal transduction in vasculogenesis and angiogenesis process. It was also supported by the antiangiogenesis effect 48 h post-labor [22].

Another function of VEGF is for growth and proliferation of glomerulus and peritubular endothelial cell [23]. VEGF inhibitor could prevent neovascularization on tumor cell, while treatment of VEGF antagonist on cancer patient would cause proteinuria and hypertension, similarly as the symptoms in preeclampsia [22-23]. Pregnant mice that were induced with sFlt-1 would show glomerular enlargement with capillary occlusion due to hypertrophy on glomerulus capillary endothelial cell. There were also protein resorption problems on podocyte [22]. These factors were the cause of hypertension and proteinuria on preeclampsia [24,25].

VEGF will influence the expression of nitrite oxide (NO). There was an increase on endothelial NO synthase (eNOS) expression on the cell that cultured and incubated with VEGF. It happened through the activation of PI3K and ERK phosphorylation [26]. When VEGF was bound to Flt-1 receptor which is a tyrosine kinase receptor, it might cause autophosphorylation of tyrosine794 residue (Tyr794). Then, PI3K will be activated, followed by AKT Activated AKT would phosphorylate eNOS cause the NO production to increase. The same effect also happened when VEGF was bound to KDR/Flk-1 receptor [27]. On preeclampsia, one of the symptoms was hypertension; the decrease of VEGF protein expression could cause vascularostriction due to the repression of NO expression. NO was main endothelial relaxation modular. It acts as paracrine and autocrine to protect cardiovascular homeostasis, tonus vascular muscle, and also microvascular permeability.

RAS or known as RAS plays a major role in pathogenesis preeclampsia. In early pregnancy, prorenin, (P)RR, and angiotensin II type 1 receptor were found on extravillous trophoblast (EVT). It explains the effect of RAS components in trophoblast migration [10]. In normal pregnancy, there was an increase of RAS components which were renin, angiotensinogen, angiotensin I, and aldosterone [28,29]. It does not happen to angiotensin-converting enzyme which its level remains the same as normal pregnancy. It explains why the increase of RAS components was not followed by hypertension in normal pregnancy [28].

(R)Prorenin receptor which is the receptor of renin and prorenin was expected to play major role in implantation process. When the ligand was bound to (P)RR, it could raise the signal of angiotensin II (ANG II)-dependent pathway and ANG II-independent pathway. It was expected to play a major role in vasculogenesis and angiogenesis process. In the research conducted in in vivo and in vitro, it was proven that prorenin
bound to (P)RR plays a role in increasing neovascularization [30]. This role was supported by the existence of (P)RR at STB and EVT, and also (P)RR mRNA expression in normal pregnancy was higher at the early pregnancy than at the term pregnancy. In this research, the (P)RR mRNA expression in preeclampsia pregnancy was higher than the normal group. On the other hand, the (P)RR protein expression was in preeclampsia was lower than in normal group. It shows that there were post-transcription arrangements in (P)RR expression. To fulfill its needs, cell tries to increase the expression of (P)RR mRNA in aiming that the protein expression would also increase. On the other hand, Thomason et al. analyzed the (P)RR protein expression in pregnant mice discovered that there was an elevation of (P)RR protein expression in preeclampsia, but there was no correlation between them [30].

(Pro)renin receptor existence is very important in placenta process. It is believed that there is a correlation between (P)RR expression and VEGF expression. A research conducted by Kanda et al. on HMRECs concluded that there was a correlation between the activity of (P)RR and angiogenesis on diabetic retinopathy. The elevation of protein concentration would increase the level of VEGF mRNA expression [11,31]. Binding of prorenin with (P)RR would stimulate ERK phosphorylation, also it will trigger an action without involving angiotsin I. The involvement of (P)RR was proved by the inactivated (P)RR that would also inactivate ERK. Inhibitor (P)RR could suppress the expression of VEGF on pregnant diabetic mice. The resistance of VEGF expression occurred through the suppression of ERK ½ phosphorylation [32,33]. This research also shows the relations between (P)RR protein expression and VEGF protein expression. There was positive correlation between normal group and preeclampsia with medium relation between (P)RR protein expression and VEGF protein expression. It proves that there was an involvement of (P)RR in VEGF expression arrangement on preeclampsia.

CONCLUSION
VEGF protein expression decreased in line with the decreasing of (P)RR protein expression on the placenta of the third-trimester pregnant women with preeclampsia. There was a correlation between the expression of (P)RR and VEGF.

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AUTHORS’ CONTRIBUTION
Author contributions were as follows: Nelly Marissa measured mRNA relative expression and ELISA of markers, Sri Widia A Jusman data analysis and correction, Yuditja Purwo susu collect the samples, and Ani Retno Prijanti searched foundation as person in charge and manuscript correction.

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