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Identification of Apoptosis Marker in Preeclampsia: A Pre-Eliminary Study of P53 Gene Expression in Human Placenta

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ABSTRACT
Preeclampsia still becomes a leading cause of maternal and perinatal mortality. The placenta is a critical subject in the pathogenesis of preeclampsia. Excessive apoptosis can cause the malfunction of trophoblast cells in their ability to remodel spiral arteries. Uteroplacental ischemia and endothelial cell injury may result in abnormal remodeling activity which may lead to preeclampsia-like symptoms. The p53 is a gene that acts as an apoptosis regulator in cells (as pro-apoptosis). This gene will cause the cascade apoptotic pathway, leading to cell death. Aim of this study to identify differences of p53 gene expression between preeclampsia and normal pregnancies. This is a case control study, performed in Biomolecular Laboratory Faculty of Medicine and Health science Universitas Jambi. Human placenta from patients with preeclampsia (n=25) and normal pregnancies as control (n=25) were collected from several hospitals in Jambi City from June 2020 to August 2020. Expression of p53 was assayed by quantitative real time PCR (qRT-PCR). Gene expression of p53 was significantly increased in pregnancies with preeclampsia (1,91± 5,90) compared to normal pregnancies group (4,85± 3,04) (t-test; p< 0.032). Expression of p53 has relatively increased by 7 fold for relative quantification by normalized with the internal control target gene GAPDH. Expression of p53 gene in preeclampsia is higher than normal pregnancies and There was significant difference between two groups in p53 gene expression.

Keywords: *P53, Preeclampsia, Apoptosis.*

1. INTRODUCTION

Within 20 weeks of pregnancy, preeclampsia is an antepartum problem in the multi-organ system characterized by hypertension and proteinuria. Preeclampsia happened in 5-8 percent of all pregnant women globally and is one of the major contributors to maternal-fetal morbidity and mortality [1]. The incidence of preeclampsia is quite high with underlying pathophysiology and exact etiology of this condition still unclear. There are several theories regarding the ultimate cause of preeclampsia, including systemic endothelial dysfunction, inflammation, and angiogenic factors, but none of these theories are considered correct ([1] [2] [3]). As a result, until now there is no definite treatment for this disorder.

Preeclampsia still has an unclear etiology and pathomechanism. Based on past research, several theories have shown an increase in pregnancy blood pressure. Some of these theories are (1) vascular disease and endothelial dysfunction in the placenta; (2) placental ischemia, free radicals; (3) immunological intolerance between mother and fetus; (4) genetic deficiency; (5) nutritional deficiency; (6) inflammation. According to the theory of placental ischemia, the pathogenesis of preeclampsia is associated between placental ischemia and endothelial cell dysfunction [4]. Pathologic of Anatomic examination of the placenta related to

preeclampsia shows placental infarcts and sclerotic narrowing of artery and arterioles [5].

In an ordinary pregnancy state, apoptosis assumes a fundamental part in the regulation of placental development. Apoptosis in the placenta increased in number along with placental development and progressing in gestational age. However, based on previous epidemiological studies in various populations, Preeclampsia, which is caused by a combination of factors, including hypoxia and oxidative stress, causes a significant increase in placental apoptosis. A decreased in the number of trophoblast cells inside the spiral arteries has been related to a reduction in lumen thickness, which leads to placental perfusion to decrease 2-3 times and also produced large pressure placental blood circulation. This process inhibits the implantation of trophoblasts, proliferation, and regulation of trophoblast development [6] [7].

Several studies about preeclampsia and apoptosis have been conducted to explore genetic markers associated with the pathogenesis of preeclampsia, but the result remains uncertain. One of the main marker components that stimulate the apoptotic signaling pathway, namely the tumor suppressor p53. In addition to genotoxic or cellular stress, the tumor protein p53 plays the role of a nuclear transcription factor, controlling the expression of numerous apoptosis-related genes. When cells experience stress such as DNA damage, hypoxia, exposure to certain cytokines, metabolic changes, viral or oncogene infections, p53 becomes stable and accumulates in the nucleus. Furthermore, p53 underwent various modifications to become an active protein. Active p53 binds as a tetramer and works as a transcription factor that activates downstream gene expression, leading to programmed cell death [8].

In our study, we investigated the differences in pro-apoptotic p53 gene expression between preeclampsia and normal pregnancy patients in Jambi City.

2. METHODS

This is a case-control study performed in the biomolecular laboratory Faculty of Medicine and Health Science Universitas Jambi. The human placentas from patients with preeclampsia (n=25) and normal pregnancies as control (n=25) were collected from several Hospitals in Jambi city from June 2020 to August 2020.

2.1. RNA isolation and cDNA synthesis

Placental tissues were completely mixed thoroughly in RNA lysis buffer with sterile pestels until being centrifuged at 14000 rpm for 10 min at 40°C to eliminate the insoluble substance. The RNA extraction kit (Promega, USA) is used according to the manufacturer's protocol to extract whole placental RNA from samples as a starting product. Approximately 7 µg of each RNA sampel were used to synthesize Complementary DNA (cDNA). cDNA synthesis were performed using a cDNA synthesis kit (Promega, USA). The reverse transcription reaction was performed on an Arktik Thermal Cycler PCR (Thermo Fisher, USA) at 25°C for 5 min, 42°C for 60 min, and 70°C for 15 min. The cDNA was synthesized and placed at 20°C before being used. The cDNAs were then used as the template for the qRT-PCR reaction to analyze gene expression.

2.2. Quantification by real-time PCR assay

Amplification of the p53 gene was performed in 20 µl reaction mixes containing the following reagents: sybr green master mix 12.5 µl (Promega, USA), 2 µl p53 forward primer, 2 µl p53 reverse primer, 0.2 µl DNA dye, nuclease-free water 2.8 µl, and 3 µl cDNA. All real-time PCR was performed using the PicoReal96 real-time PCR system (Thermo Fisher, USA) with the following setting: 1 cycle at 95oC for 10 minutes, followed by 40 cycles for the denaturation process at 95oC for 30 secs, annealing at 59oC for 10 min. Chain elongation and detection at 72oC for 30 secs. The p53 gene's relative expression was standardized using the human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a reference gene. GAPDH thermal cycling conditions are one cycle at 950C for 5 minutes, trailed by 55 cycles of denaturation at 950C for 10 seconds, annealing at 600C for 20 seconds, and extension at 720C for 10 seconds.

Table 1. Primers and sequences in real-time PCR studies

Gene Name	Forward Primer	Reverse Primer
p53	5’ GGA AGA GAA TCT CCG CAA 3’	5’ AGC TCT CGG AAC ATC TCG AAG 3’
GAPDH	5’-AGC CAC ATC GCT CAG ACA C-3’	5’-GCC CAA TAC GAC CAA ATC C-3’

2,3, Statistical Analysis

SPSS Statistics 22.0 program (IBM Corp.) was used for statistical analysis in the study of the p53 gene expression. The variations between the preeclampsia and healthy pregnancy groups were determined using an independent student T-test. The Information data was introduced as the mean ± SD and a p-value <0.05 was considered to show a level of significance. The fold change in relative expression levels of p53 was quantified using the 2-ΔΔCq methods, using housekeeping gene as references, and followed according to the manufacturer's instructions.

3. RESULTS

In the preeclampsia group (n=25), the mean level of p53 gene expression is 1.91±5.90 and in the normal pregnancy group (n=25), the mean level of p53 gene expression is 4.85±3.04. There are significant differences (p-value = 0.032) in level of p53 gene expression between two groups (figure 1). The p53 gene expression was higher in the preeclampsia group than in the normal pregnancy group. (7.686) fold overexpression (table 2).

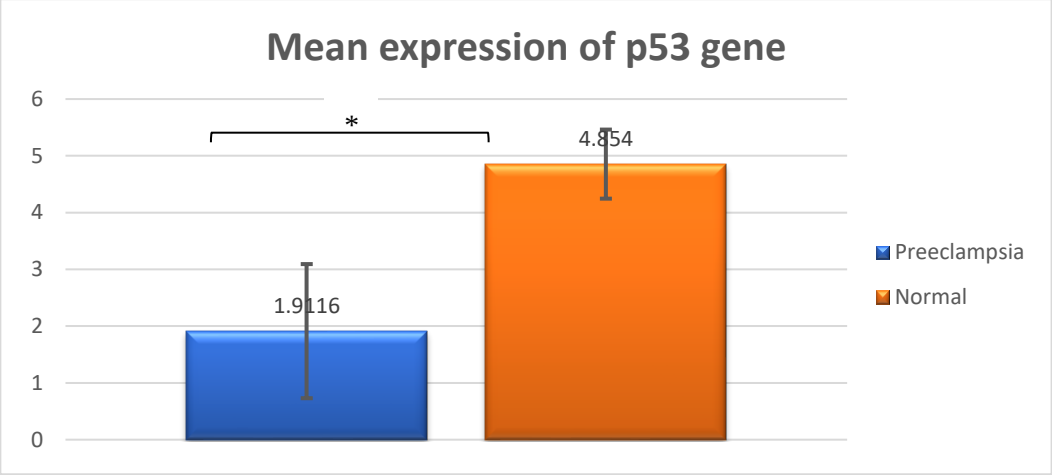

Figure 1. The mean of p53 gene expression in preeclampsia and normal pregnancies. * p < 0.05

Table 2. Relative expression of p53 gene between pregnancies with preeclampsia and normal pregnancies.

Target Gene	ΔCq (Case)[1] n=25	ΔCq (Normal)[2] n=25	ΔΔCq[3]	$2^{-(\Delta\Delta Cq)}$[4]
p53/GAPDH	1.9116	4.854	-2.9424	7.68689

[1] Δ*Cq* Case = *Cq* examined gene – *Cq GAPDH*;
[2] Δ*Cq* normal = *Cq* examined gene – *Cq GAPDH*;
[3] ΔΔCq = Δ*Cq* Case - Δ*Cq* normal
[4] $2^{-(\Delta\Delta Cq)}$ = fold change

4. DISCUSSION

An increase in pregnancy blood pressure associated with preeclampsia is developed during pregnancy and typically resolves after birth. The placenta appears to have an essential role in this condition. According to the evidence, the dysfunction of endothelial cells is the major contributor to preeclampsia. Anatomic pathology examination of the placenta related to preeclampsia shows placental infarcts and sclerotic narrowing of artery and arterioles. The other factor that triggers preeclampsia is apoptosis as a result of placental hypoxia leading to placental ischemia [4] [5].

Abnormal apoptosis in the placental has a negative impact that extends beyond the maternal placenta, most notably on endothelial cells. Placental apoptosis is constantly expanding in response to genotoxic or cellular stress [8]. Hypoxia and oxidative stress are the factors that most often stimulate p53 gene overexpression in preeclampsia [9]. Pro-apoptotic p53 gene overexpression leads to exaggerated apoptosis caused by trophoblast damage. This research found differences in the expression of the p53 gene in the placentas of pregnant women with preeclampsia and a control group with normal blood pressure.

Our data indicated that the levels of p53 gene expression were higher in 25 pregnant women with preeclampsia, compared to 25 pregnant women with normotension in the control group. There are significant differences (p-value = 0.032) in the level of p53 gene expression between two groups. p53 is one of a gene that plays a role as a cell apoptosis regulator. This gene would activate the apoptotic cascade mechanism that leading to cellular death activity. For normalization, the GAPDH gene was used as the reference gene. This result is consistent with the theory that overexpression of the p53 gene frequently occurs in preeclampsia stimulated by placental hypoxia [10] (Teguh, 2008).

Over-expression p53 gene initially promotes the transcription of p21, an inhibitor for cell cycle and affects cell-cycle arrest and autophagy. This condition causes prolonged oxidative stress which encourages apoptosis

by disrupting the ratio of pro-apoptotic such as p53, p21, Bax and anti-apoptotic such as Bcl-2 and Mcl-1 proteins [11]. The Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene encodes a crucial enzyme taking part in glycolysis for the production of energy. Recent research has found that GAPDH nuclear translocation plays an important role in apoptosis. GAPDH in the nucleus creates a protein complex with p53, which leads to increased expression and phosphorylation of the p53 gene [12]. As a response to placental tissue damages, p53 and GAPDH may be involved in preeclampsia endothelial cell dysfunction triggered by enhanced autophagy and apoptosis.

Overexpression of BCL-2 with low-level p53 gene expression is required for healthy placental development and continuity in pregnancy. Otherwise, failure in placental growth could be a result of p53 overexpression in placental tissues through an apoptosis mechanism. Elavated apoptosis in preeclampsia occurs through the intrinsic pathway in response to a stress cell. This condition initiates p53, the pro-apoptotic protein, would inhibit the anti-apoptotic gene like Bcl-2 Since p53 will increase the expression of many death receptors through the extrinsic pathway, the two pathways are linked [13][14]. Preeclampsia was associated with elevated p53 expression in the trophoblast layer, as well as the other apoptotic proteins such as p21 and Bax. Exaggerated apoptosis and syncytial degeneration are associated with an imbalance in p53 and anti-apoptotic gene expression [15] [16].

5. CONCLUSIONS

In conclusion, expression of pro-apoptotic p53 gene in preeclampsia is higher than normotensive pregnancies. Therefore, p53 gene expression can be considered as a marker in preeclampsia.

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