The Effect of Pravastatin Provision on Endotelin-1 Levels in Preeclapmsia's High Risk Patients

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Abstract

Objective: To determine the effect of pravastatin administration on endothelin-1 levels in patients at high risk of preeclampsia.

Method: There are 70 subjects in this research that consist of 34 subjects with the risk of high preeclampsia who get aspirin and 36 subjects of research to get Aspirin and Pravastatin. Subjects are given therapy started the gestational age of 12-19 weeks in 6 days until 36 weeks of pregnancy. Endhotelin-1 levels prior to administration of therapy compared with the levels of endothelin-1 after the administration of treatment.

Results: The test results show the administration of aspirin or aspirin and Pravastatin is shown to provide the changes were significant at levels of endothelin-1 after the administration of treatment. It is characterized by the decrease in the levels of endothelin-1 which were significantly ($p < 0.005$). But by assessing percentage decrease in the provision of aspirin with pravastatin give change levels of endothelin-1 is large (45.74%) compared with administration of aspirin alone (28.8%).

Conclusion: From this study it can be concluded that endothelin-1 levels before administration of aspirin and pravastatin were 86.18 ng/L and after administration of 46.76 ng/L. Giving aspirin and pravastatin 20 mg in patients with high risk factors of preeclampsia provide reduced levels of endothelin-1 is greater than the prevention of preeclampsia that is used when it is only aspirin.

Keywords: *Patient risk of high preeclampsia, Aspirin, Pravastatin, Levels of Endothelin-1.*

Introduction

Preeclampsia is a multisystem disorder in pregnancy that is characterized by hypertension and proteinuria after 20 weeks period of gestation. Abnormalities that occur in 5 stars - 7 % pregnancies, with figures incidence of 23.6 cases per 1,000 births in the United States the United t.[1] Preeclampsia is a specific pregnancy disease and is still the leading cause of maternal-neonatal mortality and morbidity worldwide, accounting for more than 70,000 - 80,000 maternal and 500,000 perinatal deaths.[2]

The imbalance of pro and antiangiogenic factors as well as the activation of mediators of immunity that contribute to excessively inflamation is a major cause. The etiology of preeclampsia is unclear, but several recent studies have shown that an imbalance between angiogenic factors (*Vascular Endothelial Growth Factor* (VEGF) and *Placental Growth Factor* (PLGF)) and anti-angiogenic (*Soluble fms-like Tyrosine kinase 1* (sFlt-1) and *Placental Growth Factor* (PLGF)) and anti-

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angiogenic (*Soluble fms-like Tyrosine kinase 1* (sFlt-1) and *Placental Growth Factor* (PLGF)) *soluble endoglin* (s-eng)) plays an important role in the pathogenesis of this disease.[2]

Endothelin 1 (ET-1) is an isoform primary that produced by endothelial humans and peptide 21-amino-acid is a vasoconstrictor potent. Levels of ET-1 in plasma increased in pregnant women normotensive, but women with preeclampsia had levels of ET are much higher. Endothelin-1 has proven be the holder of the key to the activation signal molecular that caused stress oxidative in patients with preeclampsia that inhibits the release of ET-1 is one of the alternative in preventing the occurrence of preeclampsia.[3]

The International Study Collaboration conducted a meta- analysis of the effectiveness of antiplatelet agents (especially aspirin) for the prevention of preeclampsia. Low- dose aspirin (60-150 mg per day) can reduce the risk of preeclampsia by 24%.[4]On the other, Ahmed et al propose a statin as a new therapeutic strategies in which case it is based on studies of experimental animals showed that pravastatin (inhibitor coenzyme-A reductase 3-hydroxy-3-methilglutaril) has a protective role in the uteroplacental circulation and blood vessel cells.[5]

When this, pravastatin has demonstrated the effect of protection against endothelial cells vaskular induces the expression of H eme O ksigenase (H O -1) and inhibits cytokine release from anti-angiogenic factor sFlt-1 and zinc. Increased levels of sFlt-1 and zinc causes a decrease in VEGF and PlGF free on placental cells and endothelial vessels of blood which ultimately resulted in systemic vasoconstriction and endothelial dysfunction vessels of the blood of the mother.

Therefore, the purpose of the research is to determine the effect of administration of aspirin and pravastatin on the levels of endothelin - 1 in patients Be risk of high preeclampsia .

Material and Method

Design of study: This study was a quasi-clinical trial with randomization, which carried out clinical trials on control groups and cases during the 1- year study period.

Population of study: Samples of research are taken from a woman pregnant at high risk of preeclampsia with age pregnancy 12 weeks - 19 weeks 6 days of the visit to the clinic of the General Hospital of Makassar and Jumpandang Baru health care center. Sampling was carried out by block randomization in the administration of therapy. The provision of interventions carried out *single blinding* that participants can determine the allocation of the group based on the amount of drug that is given.

Study Interventions: This study was conducted to assess the effect of giving pravastatin to pregnant women who are at high risk of preeclampsia. Subject tests are given aspirin 1 x 80 mg orally until the age of term pregnancies as a treatment standard (group A), while the group of samples are given intervention in the form of aspirin 1 x 80 mg orally plus pravastatin who consumed 2 times a day until the age of term pregnancies in the same time (group B).

Randomization: If the patient agrees to be a participant in this study, it is mandatory to sign the research consent sheet. Participants will then be randomized into two groups: those who receive a combination of pravastatin and aspirin, and those who receive aspirin only. Participants will take the drug until the end of pregnancy Endothelin-1 levels will be checked before and after drug administration.

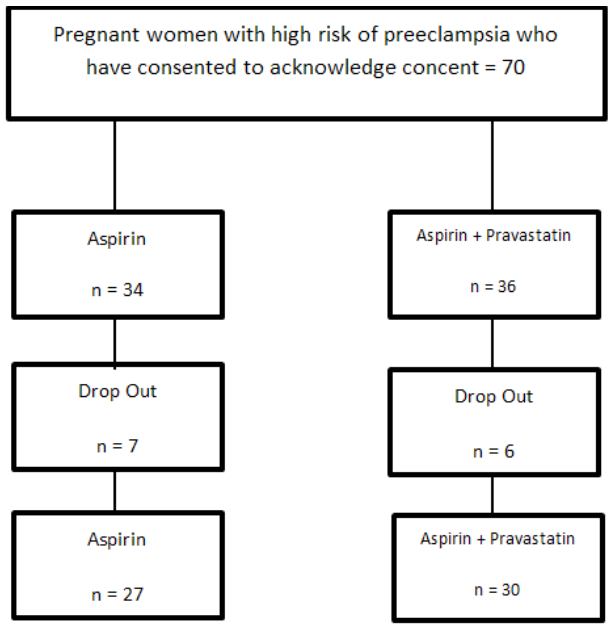

Figure 1. Flowchart of screening of participans

Study Outcomes: Results of the study is expected to provide information about the profile levels of endothelin - 1 on a mother pregnant as well as the effect of giving aspirin and pravastatin on the levels of endothelin - 1 in patients Be risk of high preeclampsia .

Sample Size: In the study of this, a large sample in each group of the intervention and the control is determined by a formula as follows

$$n1 = n2 = \left[\frac{(Z\alpha + Z\beta)S}{X1 - X2}\right]^2$$

N is total samples required for each intervention and control groups . Zα is a type I error, which is 5% with a one-way hypothesis with a constant value of 1.64 . Zβ is k esalahan type II set at 10% by value of the constant 1.28 . S is s impang raw (Verdonk et al., 2015) with a value of 0.42 . X1 - X2 is s elisih minimum considered significant at 0.21 . So the value of n in this study are:

$$n1 = n2 = \left[\frac{(1.64+1.28)0.42}{0.21}\right]^2 = 34$$

Statistical Analysis: Data were collected and have been analyzed and then processed by computer using SPSS. Normality test was performed with Kolmogorov-Smirnov (samples> 50). If a normal distribution is obtained (p> 0.05) a paired T test is used and if an abnormal distribution is obtained (p <0.05) the Wilcoxon test is used to compare the average serum Endothelin - 1 level before and after drug administration in each each sample and control group . Then if a normal distribution is obtained (p> 0.05) an unpaired T hypothesis test is used and if an abnormal distribution is obtained (p <0.05) the Mann-Whitney test is used to compare the average difference in serum Endothelium - 1 levels between sample groups with a control group.

Ethical Approval: Before the study is conducted, the researcher asks the feasibility of conduct of the Commission of Ethics Research Biomedical on Human Faculty of Medicine, University of Hasanuddin Makassar and has been approved by the number of register 601/ H4.8.4.5.31/PP36- KOMETIK/2018 by chairman Prof. Dr. dr. Suryani As'ad, M.Sc., Sp.GK and secretary Dr. Agussalim Bukhari, M.Med ., Ph.D., Sp.GK .

Results

Samples Characteristics:

Table 1. Demographic Characteristics of Research Subjects.

Variable	Aspirin (n = 34)		Aspirin + Pravastatin (n = 36)		P value
	n	%	n	%	
Age					
<20 years old	0	0	0	0	
20 - 35 years old	17	50	23	62.9	0.469 [a]
> 35 years old	17	50	13	37.1	
Parity					
• 0 children	3	8.8	7	19.4	0.734 [a]
• ≥ 1 child	31	91.2	29	80.6	
Education					
• Basic education	15	44.1	22	61.1	0.15 [b]
• Further education	19	55.9	13	36.1	
Body Mass Index (BMI)					
• Normal	7	20.6	8	22.7	
• Overweight	9	26.5	6	16.2	0.643 [a]
• Obesity	18	52.9	22	61.1	

a: *chi-square* test, b: *mann-whitney* test

Table 2. Risk Factors Research subjects.

Variable	Aspirin (n = 34)		Aspirin + Pravastatin (n = 36)		P value
	n	%	n	%	
Diabetes					
• Yes	0	0	0	0	-
• Not	34	100	36	100	
Kidney failure					
Yes	0	0	0	0	-
• Not	34	100	35	97.2	
Hypertension					
• Yes	11	32.4	7	19.5	0.243 [a]
• Not	23	67.6	29	80.5	
Gestational Diabetes					
• Yes	0	0	0	0	-
• Not	34	100	36	100	
History of Preeclampsia					
Yes	13	38.2	14	38.8	0.881 [a]
• Not	21	61.8	25	61.2	

By follow up, there were 1 or 3 subjects who *dropped out* for various reasons.

Differences Endothelin-1 se is not the provision of treatment

Table 3. Differences in Endothelin-1 before and after administration of aspirin alone with aspirin and pravastatin.

Variable	Aspirin + Pravastatin (Mean±SD)	Aspirin (Mean±SD)	P value
Endothelin-1 Pre- intervention	86.18±129.46	109.61±199.33	0.948 [b]
Endothelin-1 Post Intervention	46.76±95.1	78.04±166.97	0.655 [b]

b: *mannwhitney* test

Changes Endothelin-1 prior to and throughout has been the provision of treatment:

Table 4. Changes in Endothelin-1 levels before and after administration of aspirin alone with aspirin and pravastatin

Variable	Endothelin-1 Pre-intervention (Mean±SD)	Endothelin-1 Post Intervention (Mean±SD)	Percentage of Decrease (%)	P value
Aspirin + Pravastatin	86.18±129.46	46.76±95.1	45.74	0.008 [c]
Aspirin	109.61±199.33	78.04±166.97	28.8	0.046 [c]

C: *Wilcoxon* Test

Based on *mannwhitney* test, table 3 shows the p-value have no differences s of endothelin-1 level before and after therapy in both groups of treatment ($p > 0.05$). The intervention that is given to the subject of research at the given on samples with endothelin pre-intervention were homogenous. So the intervention is given in the sample with endothelin start of the homogeneous.

Based on Wilcoxon test, the administration of aspirin or aspirin and pravastatin shown to provide the changes were significant at levels of endothelin-1 after the administration of treatment . It is characterized characterized by a decrease in the levels of endothelin-1 were significantly ($p < 0.005$). But by assessing the percentage decrease in the provision of aspirin with

pravastatin give change levels of endothelin-1 is large compared with the administration of aspirin alone.

Discussion

Preeclampsia is a specific hypertensive disease in pregnancy which involving multisystem. Usually, after gestation age of 20 weeks, most often in the near term, can be superimposed with other hypertensive disorders. Preeclampsia, the most common form of high blood pressure (*BP*) which could complicates pregnancy, is mainly determined by the incidence of new onset hypertension and the presence of new onset proteinuria. However, although both of these criteria are regarded as classical definitions of preeclampsia, some women come with hypertension and multi-systemic signs that usually indicate the severity of the disease without the presence of proteinuria.[6]

If proteinuria is not present, the diagnosis of preeclampsia is enforced when there is hypertension with thrombocytopenia (plateletcount less than 100,000 per microliter), the presence of impairedliver function (increased levels of liver transaminases in the blood totwo times from the normal concentration), the new development of renal insufficiency (increasecreatinineserum greater than 1.1 mg/dl or doubling thecreatinine serum with outany other kidney disease), the presence of a lung edema,or a brain disorder or new onset visual impairment.[6]

Table 2 showed that both the sample and the control Group have the most age in the 20 – 35 years old, in which the sample Group consist of 23 Samples (62.9%) and 17 sample for the control Group (50%). In the Sample group, the most parity count is ≥ 1 as many as 29 Samples (80.6%) and similarly to the control group as many as 31 Samples (91.2%). Further, most samples's education in sample group were elementary which is 22 Samples (61.1%) and the control group found that most of the samples at the advanced education were 19 Samples (55.9%). Then, based on body mass index (BMI) both the sample and the control was found mostly in obese condition which are 22 Samples (61.1%) and 18 Samples (52.9%) respectively.

In particular in table 3 only obtained risk factors with a history of hypertension and previous preeclampsia history in sample groups as much as 7 Samples (19.5%) and 14 samples (38.8%) as well as in a control group of 11 samples (32.4%) and 13 samples (38.2%).

Based from table 1 and table 2, the age, the number of parity, the education status, the body mass index and the previous history of the disease such as hypertension and preeclampsia was not found a meaningful differences in both Research groups, where the value of p > 0,005 on each research variable. This finding indicating that both groups have the characteristics of respondents who are homogeny.

In this study, endothelin-1 levels were the aim. Thus, the study does not found meaningful differences on the levels of endothelin-1 before and after therapy in both groups of treatment. Even if theendothelin levelon the control group(aspirin) much higher than the treat group (aspirin + pravastatin), however, the levels are not so much distinct. This is due to a spread of data that may be too broad due to the range in the sample rate because of the difficulties to control several factors due to the research limitations namely race, ethnic, dietary and previous history of illness. This is evidenced by the unusual data spread from the beginning in both groups in statistical tests. Based on a study by Rahman Shah (2007) endothelin-1appears to have a diverse role as a vascular and growthmodulator and as a mediator in many cardiovascular and non-cardiovascular diseases. ET-1 is the primarily a paracrine substance that acts locally which appears to contribute to the maintenance of basal vascular tone. Moreover, it is also activated in some diseases, including congestive heart failure, arterial hypertension, atherosclerosis, endothelial dysfunction, coronary artery disease, renal failure, cerebrovascular disease, pulmonary arterial hypertension, and sepsis. The increased concentration of *ET-1* can be attributed to increased activity of endotheline-modifier enzymesand to indicate endothelial damage in the circulation of pregnant women with preeclampsia.

After the intervention was found a decreased level in endothelin-1 for both groups with aspririn or pravastatinintervention which there was decreased levels of endothelin-1 by 28.8% in the Control Group (aspirin) pre- intervention from 109.61±199.33 to 78.04±166.97. However, the decrease in endothelin-1 in the sample group (aspirin + pravastatin) is greater than 45.74% from the rate of 86.18±129.46 to 46.76±95.1 after intervention. Based on the findings of this study was observed that both the administration of aspirin and aspirin and pravastatinom could reduce the levels of endothelin-1. Nonetherless, due to the absence of normal value from Endothelin-1, it is not yet clear whether the decrease in the rate is up to normal value or only decrease

its level from the initial rate. This is one of the limitation of this study. This is in line with the research conducted by Raditya Ery Pratama, dkk(2018) in Surabaya which proves that the combination of pravastatinom and standard therapy lowers Endothelin-1 levels.

This research is able to provide data on Endothelin-1 levels after aspirin therapy in high-risk preeclampsia groups. Further, this data shows that there is a decrease in the level of endothelin-1in the group of aspirin but the level of endothelin-1 after therapy is quite relatively high. The data can be an initial input, that perhaps the aspirin is not good enough to suppress endothelin-1 in order to prevent preeclampsia, as it is known that the preeclamicpatomecanism is multifaktorial, not only caused by the increasing in endothelin-1.

The results of this research are as well as the study conducted by Costantine*et al* for *the Eunice Kennedyshriver National Institute of Child Health and Human Development Obstetric-Fetal pharmacology Research Units Network (OPRU),* where it publishes a pilot of a multicenter, *double-blind*, placebo-controlled test, random of the woman with a single pregnancy with gestation age 12 to 16 weeks which randomly receives either 10 mg of pravastatindaily (n = 10) or placebo (n = 10).[7] Four women in the group given the placebo had high preeclampsia with a concentration of *sFlt-1 (soluble fmslike tyrosine kinase-1);* Three of them showed severe conditions compared to the pravastatin given group. Thus, pravastatinshows beneficial benefits in preventing the occurrence of preeclampsia.[7] In addition, there are also numbers of preeclampsia incidence and lower premature births, concentration of sFlt-1 and sENG (Endotelin) decreased in subjects who administered pravastatin. Although the difference for these results does not achieve statistical significance.[8]

Another study by Hassanain*et al* (2018) reported 4 patients having significant hypertension (90-105/155-200 mm Hg) with proteinuria ranging from 840 to 2990 mg/24 hours later administered daily pravastatin(40 mg) since the first day of admission Hospital.[6] Then in line with the effects of proangiogenic pravastatin, little but not significantly lowered the anti-angiogenic factor *sFlt-1 (soluble fmslike tyrosine kinase-1)* and $_{S}$*ENG (soluble Endoglin*) which was observed in the group given pravastatincompared to placebo group.[6]

For Evidence-Based Preeclampsia Prevention found that therapy with 150 mg of aspirin in high-risk women subjected to preeclampsia (based on algorithms that utilize maternal factors and biophysical and biochemical measurements) that began at gestation age 11 to 14 weeks lowered preeclamsia incidents compared to women who received placebo.[9]

Apart from the data limitation of aspirin, in 2013, the *American College of Obstetricians and Gynecologists (ACOG)* recommends administering daily aspirin (80-100mg) for women with preeclampsia history in previous pregnancies before gestation 34 weeks age or preeclampsia history in the previous two pregnancies.

Low-dose Aspirin given to pregnant women with high risk of preeclampsia may decrease preeclampsia incidence of up to 10-24%, lowering the incidence rate of IUGR by 20%, and premature delivery by 14%. However studies have shown that low dose aspirin administration does not lower the preeclampsia incidence at gestation age more than 37 weeks.[10] Similarly, other studies, stating that low-dose aspirin is useless to prevent preeclampsia in patients with chronic hypertension.[7] Therefore, it is preferred to give pravastatinom to decrease the incidence of preeclampsia.

This study shows the administration of aspirin and Pravastatinand aspirin proved to provide significant changes in endothelin-1 levels after administration of therapy is in the Group ofPravastatinom ($P < 0.05$, $p = 0.008$) and on the control group (Aspirin) ($P < 0.05$, $p = 0046$). However, a greater reduction in endothelin-1 levels was given to the group of pravastatin. Therefore, pravastatinis more preferred as a therapy to lower endothelin-1 levels in prevention of preeclampsia cases.

Some recent studies have also added pravastatinas an agent other than low dose aspirin to prevent preeclampsia. Pravastatin is chosen due to the basis of preeclampsia pathogenesis having similarities with cardiovascular disease i.e. systemic endothelial dysfunction.

Pravastatin is chosen from various other statins drugs due to its hydrophilic properties. Its protein binding is the lowest therefore drug interactions are also minimal (Lecarpentier*et al*, 2012). Oral absorption is about 40-75%, passing the first metabolic rate in the liver. Excretion of pravastatinin the form of intact through the liver and kidneys (secretion in tubule). The average absorption time is 2.4 hours with a bioavailability of 18%. The time to reach the maximum concentration in plasma averages one hour after oral administration.[11]

Statins act pleiotropic on various mechanisms: reverse angiogenic imbalance by increasing vascular endothelial growth factor (*VEGF*) and placental Growth Factor (PLGF), and reduce antiangiogenic factors such as *soluble fmslike tyrosine Kinase-1 (sFlt-1)* and *soluble Endoglin (sENG)*.[6]

Pravastatin is a competitive inhibitor of a *3 hydroxy-3-methyl-glutaryl-coenzymeA*reductase which is an enzyme catalyzing step limiting levels in the synthesis of cholesterol. Pravastatin is the most hydrophilic polar compound among current statins and is therefore considered to have limited ability to traverse the placenta. It is also the most powerful inhibitor of sterol synthesis.[6]

All of the biological effects of pravastatin can restore maternal endothelial dysfunction so that maternal vascular reactivity can be reduced. It can prevent maternal systemic vasoconstriction so that blood pressure will not increase.[11]

Ethical Clearance: No ethical approval is needed.

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Conflict of Interest: Nil

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