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The Difference in Endothelial Progenitor Cells (Epcs Cd34), Clotting Factors, Blood Pressure and Proteinuria among Preeclampsia Model Mice with and without the Administration of Epcs Cd34

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Background: Preeclampsia is the leading cause of mortality in 15-20% of pregnant women worldwide. EPC is part of a hematopoietic stem cell marker molecule, namely CD34. EPC have a strong relationship with the improvement of endothelial function and blood angiogenesis process. Until now, there are not many investigations regarding the potential of EPC for therapy for preeclampsia, although it is known that in people with preeclampsia the number of EPC has decreased. The use of EPCs as an alternative to endothelial cell regeneration in the future has enormous potential in its use as modern therapies.

Objective: To determine the differences in CD34 EPC counts, platelet aggregation levels, fibrinogen, D-dimer, PAI-1, blood pressure, and proteinuria among preelamptic model pregnant mice with and without administration of EPCs CD34.

Methods: This research method is pure experimental using 3 stages of research analyzed using unpaired t test or Mann Whitney test if the data is not normally distributed. All research data will be analyzed using SPSS version 22 software.

Research result: In this study, the average number of CD34 ECPs in the group given EPCs CD34 was higher (7.334 ± 0.891 vs 7.040 ± 0.473), p value > 0.05. The average platelet aggregation in the group given EPCs CD34 was lower (27.61 ± 8.93 vs 43.56 ± 1.38), p value <0.05.Fibrinogen levels in the group given EPCs CD34 were lower (1.102 ± 0.065 vs 0.944 ± 0.074), p value <0.05.D-Dimer levels in the group given EPCs CD34 were higher (7,778 vs 2,266), p value <0.05.PAI-1 levels in the group given EPCs CD34 were higher (4.168 ± 0.564 vs 3.565 ± 0.802), p value > 0.05. Systolic and diastolic blood pressures in the group given EPCs CD34 were lower (89.8 ± 8.9 vs 187.0 ± 25.0) and (72.3 ± 10.8 vs 174.7 ± 34.4), p value <0.05.Proteinuria in the group given CD34 EPCs was lower (+4 vs +1) , p value <0.05.

Conclusion: In the preeclamptic pregnant mice group given EPCs CD34: There was no difference in the number of EPCs CD34 & levels of PAI-1, but levels of platelet aggregation, fibrinogen, blood pressure, & proteinuria in the group of preeclamptic pregnant mice that were given EPCs CD34 were lower, while D-dimer levels in the preeclamptic pregnant mice group given EPCs CD34 were higher than those in the preeclamptic pregnant mice group that were not given CD34 EPCs.

Keywords: Preeclampsia, CD34 EPCs, Platelet Aggregation, Fibrinogen, D-Dimer, PAI-1, blood pressure, proteinuria

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Introduction

Preeclampsia is hypertension with proteinuria that occurs after the 20th week of pregnancy to the 6th week after delivery. Preeclampsia is the leading cause of 15-20% of maternal mortality worldwide and the leading cause of fetal mortality and morbidity. There are more than 4 million pregnant women worldwide who experience preeclampsia each year. And every year, an estimated 50,000-76,000 women die and 500,000 babies die because of preeclampsia.[1-3]

The World Health Organization (WHO) reports that the incidence of preeclampsia in the world is still quite high. Based on the WHO report in 2014, the incidence of preeclampsia was 861 cases out of 96,494 pregnant women. In America and Europe, the incidence of preeclampsia is about 0.5% of all pregnancies, while in Asia it reaches 10-15% of all pregnancies. Indonesia is one of the developing countries with the third highest maternal mortality rate (MMR) and perinatal mortality in ASEAN and the second highest in Southeast Asia.[4]
The Indonesian Demographic and Health Survey in 2012 showed that the Maternal Mortality Rate (MMR) in Indonesia due to preeclampsia was ranked second and soared higher than in 2007, reaching 24%. Based on data reported by Dr. HasanSadikin (RSHS), in 2015 the incidence of preeclampsia was around 10%. In 2018 the number of cases of preeclampsia was 499 people, in 2019 it was 918 people, and this figure continues to increase, especially in 2018 to 2019.[5,6]

Research on preeclampsia has been carried out since decades ago, but the cause of preeclampsia is still not known certainly. Various mechanisms to explain the cause have been proposed, but still unsatisfactory, because of many theories that explain the etiology and pathophysiology of preeclampsia, preeclampsia is called "the disease of theories".[7]

Recently, it is suspected that endothelial dysfunction plays a role in the etiology and pathophysiology of preeclampsia. Endothelial dysfunction is characterized by a decrease in the ability of the endothelium to carry out homeostatic functions such as regulating vascular smooth muscle cell tone for relaxation and contraction, controlling the production of prothrombotic and antithrombotic, fibrinolytic and antifibrinolytic components.[8-11]

Disorder of angiogenesis process at placentation stage in early pregnancy can cause various disorders in pregnancy and that is preeclampsia with all its complications. Due to a disorder in spiral artery remodeling, placental ischemia occurs which will result in a disruption in the balance of proangiogenic and antiangiogenic factors which in return will lead to endothelial dysfunction. [8-11]

Endothelial Progenitor Cells (EPCs) are components that play a role in the process of endothelial formation. Endothelial Progenitor Cells (EPCs) are part of stem cells that are more mature and unipotent and the cells have the ability to divide and differentiate into endothelial cells. Endothelial Progenitor Cells (EPCs) consist of heterogeneous group of cells present in the bloodstream, originating from bone marrow and blood vessel walls which play a role in the development of vascular endothelium and hemostasis. Endothelial Progenitor Cells (EPCs) that found in bone marrow or circulating in blood vessels have been shown to have a strong relationship with the improvement of endothelial function and the process of blood vessel angiogenesis.[12-15]

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Endothelium is a layer of cells lining in the vascular wall facing the lumen and attached to the subendothelial tissue which consists of collagen and various glycosaminoglycans including fibronectins. Endothelial functions is to regulate vascular tone, prevent thrombosis, regulate the activity of the fibrinolysis system and regulate vascular growth.[2]

Endothelial dysfunction can lead to an imbalance between prostacyclin (PGE2) and thromboxane (TXA2). Prostacycline (PGE2) is a strong vasodilator and also inhibits platelet aggregation which can increase uteroplacental blood circulation. Meanwhile, TXA2 which is produced mainly by platelets, is a vasoconstrictor and also increases platelet aggregation. In preeclampsia there is a decrease in PGE2 and an increase in TXA2, resulting in vasoconstriction and reduced blood flow and increasing activation of platelets and coagulation factors.[16,17]

The coagulation system will be activated by thromboplastin which is released due to tissue damage, this will result of thrombin and plasma circulating in the blood circulation. Thrombin breaks down fibrinogen to form fibrinopeptides A and B and fibrin monomer (soluble fibrin). The monomer fibrin will then undergo polymerization to form fibrin polymer (insoluble fibrin) and activated by factor XIIIA to form a cross-linked fibrin that circulating in the circulation to form a thrombus (thrombosis process) in the microvascular and macrovascular, so that it will interfere with blood flow and cause peripheral ischemia. Endothelial dysfunction also causes the non-thrombogenic surface to turn thrombogenic, so that coagulation activation can occur. One way to determine the presence of thrombosis in the blood vessel circulation is to measure the D-dimer level, which is the result of cross-linked fibrin degradation.[16,17]

Fibrinogen also plays a role in the hemostasis system, both as a support (together with the Von Willebrand factor) in the interaction between platelets, so aggregation will occur or as a stabilization process in platelet aggregation, that occurs after the conversion of fibrinogen to fibrin and circulating in the circulation to form thrombus in the microvascular and macrovascular levels, thereby disrupting the blood flow and caused peripheral ischemia that can result in organ damage.[18]

The endothelium also plays a role in the fibrinolysis system through the release of tissue plasminogen activator (tPA) which activates plasminogen into plasmin. However, the endothelium also synthesizes plasminogen activator inhibitor-1 (PAI-1) which inhibits tPA. If endothelial dysfunction occurs, there may be an increase in PAI-1. The increase in PAI-1 causes the buildup of fibrin deposits, impaired production of anticoagulants and disorders of the fibrinolysis system.[2,19]
In preeclampsia, HELLP syndrome (hemolysis elevated liver enzymes and low platelet count) is an acute development that is life-threatening condition for the mother and baby, and which can continue until coagulopathy or disseminated intravascular coagulopathy (DIC) occurs. Disseminated intravascular coagulopathy (DIC) is a hematological disorder in which the clotting process occurs along with bleeding due to fibrinolysis. Coagulopathy and progressive conditions occurred in DIC, so it needs early diagnosis, treatment and appropriate management to reduce maternal and infant mortality and other complications. Disseminated intravascular coagulopathy (DIC) is a complication of preeclampsia that can cause death in pregnant women. It is important to know the changes that occur in the maternal hemostasis system. In pregnant women with preeclampsia, there can be an increase in platelet aggregation levels, D-dimer, PAI-1 and a decrease in fibrinogen levels compared to normal pregnant women.[1,20-37]

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Endothelial Progenitor Cells (EPCs) are part of mononuclear cells (MNCs) which have a hematopoietic stem cell marker molecule, that is CD34, a glycoprotein that mediates the attachment of EPCs to the extracellular bone marrow matrix. Phenotype characteristics of EPCs can be seen in the presence of CD34 molecules on the cell surface. Until now, research on the potential of EPCs for therapy in preeclampsia is still limited, although it is known that in people with preeclampsia the number of EPCs has decreased. The use of EPCs as an alternative to endothelial cell regeneration in the future has enormous potential as a modern therapies. Therefore, this study was conducted to determine the potential of giving EPCs in repairing vascular endothelial cell damage with the parameters of CD34 EPCs, platelet aggregation levels, fibrinogen, D-dimer and PAI-1 in preeclampsia in vivo of pregnant mice model with preeclampsia. Some researchers have tried to provide preeclampsia treatment before clinical symptoms appear in experimental animals. Experimental animal studies based on the considerations that it will be difficult in humans to see the abnormalities that occur before clinical symptoms appear during the second trimester. In this case, the use of experimental animal model research subjects can find out the problems that cannot be explained in humans due to ethical issues. The use of mice as experimental animal models are most often used in biomedical research because they are genetically similar to humans and have the ability to adapt to life in a laboratory environment.[38-46]

The primary problems that can be taken in this study are:Preeclampsia is a disorder that occurs in higher risk pregnant women, because it is still the main cause of maternal and perinatal morbidity and mortality in Indonesia. Until now, there is no definite theory of the main cause of this disease. One of the theories stated that, endothelial dysfunction play an important role in the occurrence of preeclampsia. Endothelial dysfunction causes a disruption in the balance of coagulation and fibrinolysis in the maternal hemostasis system.

Endothelial dysfunction results from EPCs balance disorder. Endothelial Progenitor Cells (EPCs) can be observed in the presence of marker molecules, such as CD34. Compared with normal pregnant women, preeclampsia patients have disorders of coagulation and fibrinolysis, causing a higher levels of platelet aggregation, D-dimer, PAI-1, fibrinogen levels, blood pressure, and proteinuria. The results showed that the number of CD34 EPCs in pregnant women with preeclampsia was lower than normal pregnant women. Until now, there are no biomarkers or markers that have been proven to be the most reliable to become a marker for early prediction of preeclampsia and as an effective therapy for treating preeclampsia. Meanwhile, if it is not early detected and is not treated properly, preeclampsia can threaten the life of the mother and the baby. Therefore, the use of EPCs as an alternative to endothelial cell regeneration in the future has enormous potential as a modern therapy. Endothelial Progenitor Cells (EPCs) CD34 can be a parameter when performing endothelial cell repair therapy, angiogenesis and perfusion of placental development in preeclamptic patients.

METHODS

This research method is pure experimental. This research was conducted in the experimental animal laboratory Faculty of Veterinary Medicine UNAIR, Installation of Tisse Bank and Cells Dr. Soetomo Hospital, Surabaya; Biology Service Unit, Faculty of Science and Technology, Airlangga University, Physiology Laboratory, Faculty of Medicine, Brawijaya University. This research was conducted from November 2019 until the sample size was fulfilled.

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The research subjects were 12 female mice that were mated before, so that they were pregnant, then they were divided into two groups, that were model of pregnant mice with preeclampsia (blood pressure> 140 / 90mmHg with proteinuria) that were not given with EPCs CD34 and model of pregnant mice with preeclampsia (blood pressure> 140/90 mmHg with proteinuria) that were given CD34 EPCs. Blood samples from mice were then taken to measure the number of EPCs, platelet aggregation levels, fibrinogen levels, D-dimer levels and PAI-1 levels. In addition, blood pressure measurements and proteinuria checks were also carried out in mice.

The research data were analyzed to determine the differences in the number of CD34 EPCs, platelet aggregation levels, fibrinogen, D-dimer, PAI-1, and blood pressure in the two treatment groups, using unpaired t test or Mann Whitney test, if the data were not distributed normally. The data first were analyzed using the data normality test using Shapiro Wilk because the sample size was <50. All research data will be analyzed using SPSS version 22 software.

The research was conducted after obtaining approval from the Promoter Team and ratification from the Research Ethics Committee (Number 327 / UN6.KEP / EC / 2019) UNPAD Faculty of Medicine UNPAD, and permission for the place to carry out the research.

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RESULTS

This research took place from November 2019 and until the number of samples were fulfilled. The study was conducted by dividing preeclampsia pregnant
mice into two groups, namely model of pregnant mice with preeclampsia that were not given EPCs CD34 and model of pregnant mice with preeclampsia that were given EPCs CD34.

Baseline Data of Research Results About the Effect of Giving EPCs CD34 on the Variables that studied

Number	Groups	*EPCs* CD34 (ng/ml)	Platelet Aggregation (%)	Fibrinogen (mg/ml)	D-Dimer (ng/ml)	PAI-1 (ng/ml)	Blood Pressure (mmHg)	Proteiuria
	I							
1	K1	6.768	43.210%	1.043	1.394	3.327	172/164	+4
2	K2	6.400	43.662%	1.019	1.415	2.216	195/183	+4
3	K3	7.806	45.902%	1.189	3.769	3.634	225/225	+4
4	K4	6.928	41.667%	1.146	3.117	4.670	191/172	+4
5	K5	7.198	43.860%	1.079	1.388	3.892	190/185	+4
6	K6	7.143	43.077%	1.134	3.512	3.654	151/119	+4
	II							
1	P1+*EPC*	6.821	11.111%	1.019	4.525	3.832	93/89	+1
2	P2+*EPC*	7.198	36.842%	1.043	8.439	3.812	99/78	+1
3	P3+*EPC*	8.493	28.571%	0.870	5.735	4.200	96/59	+1
4	P4+*EPC*	7.750	32.353%	0.870	7.418	5.185	76/66	+1
5	P5+*EPC*	5.939	25.532%	0.953	8.591	4.347	82/76	+1
6	P6+*EPC*	7.806	31.250%	0.911	8.139	3.634	93/66	-

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were given EPCs CD_{34}

Comparison of ECPs CD34 total that studied in two different groups

Variable	**Groups**		**P Value*)**
	I (n=6)	**II (n=6)**	
EPCs CD34			
Mean (SD)	7,040 (0,473)	7,334 (0,891)	0,492
Range	6,40 – 7,806	5,939 – 8,493	

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

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The average number of ECPs CD34 in the group that was given EPCs CD34 was higher than the group that was not given ECPs CD34 (7.334 ± 0.891 vs 7.040 ± 0.473) in this study. However, from t test analysis, found that there was no significant difference in the total of ECPs CD34 between the two groups (p> 0.05).

Comparison of platelet aggregation that studied in two treatment groups

Variable	Groups		P value*)
	I (n=6)	II (n=6)	
Plateletaggregation			
Mean (SD)	43,56 (1,38)	27,61 (8,93)	**0,001**
Range	41,67 – 45,90	11,11 – 36,84	

• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

The average platelet aggregation in the group that was given EPCs CD34 was lower than the group that was not given ECPs CD34 (27.61 ± 8.93 vs 43.56 ± 1.38) in this study. Based on the t test analysis, there was a significant difference in platelet aggregation between the two groups (p <0.05).

Comparison of fibrinogen level that studied in two treatment groups

Variable	Groups		P value*)
	I (n=6)	II (n=6)	
Fibrinogen			
Mean (SD)	1,102 (0,065)	0,944 (0,074)	**0,003**
Range	1,019 – 1,189	0,87 – 1,043	

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

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The mean fibrinogen level in the group that was given EPCs CD34 was lower than those that was not given ECPs CD34 (1.102 ± 0.065 vs 0.944 ± 0.074) in this study. The t test showed a significant difference in fibrinogen levels between the two groups ($p < 0.05$).

Comparison of D-Dimer level that studied in two treatment groups

Variable	Groups		P value*)
	I (n=6)	II (n=6)	
D-Dimer			
Median	2,266	7,778	**0,002**
Range	1,388 – 3,769	4,525 – 8,591	

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

The average D-Dimer level in the group that was given EPCs CD34 was higher than in the group that was not given ECPs CD34 (7.778 vs 2.266) in this study. From the Mann-Whitney test, it was found that there was significant difference in D-Dimer level between the two groups ($p < 0.05$).

Comparison of systolic blood pressure that studied in two treatment groups

Variable	Groups		P value*)
	I (n=6)	II (n=6)	
Systolic blood Pressure (mmHg)			
Mean (SD)	187,0 (25,0)	89,8 (8,9)	**<0,001**
Range	150 – 225	76 – 99	

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

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The average systolic blood pressure in the group that was given EPCs CD34 was lower than in the group that was not given CD34 ECPs (89.8 ± 8.9 vs 187.0 ± 25.0) in this study. Analyzed with t test, it was found that there was a significant difference in systolic blood pressure between the two groups ($p < 0.05$).

Comparison of diastolic blood pressure that studied in two treatment groups

Variable	Groups		P Value*)
	I (n=6)	II (n=6)	
Diastolic Blood Pressure (mmHg)			
Mean (SD)	174,7 (34,4)	72,3 (10,8)	**<0,001**
Range	119 – 225	59 – 89	

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}
• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

The average diastolic blood pressure in the group who was given EPCs CD34 was lower than the group that was not given CD34 ECPs (72.3 ± 10.8 vs 174.7 ± 34.4) in this study. From the t test, it was found that there was significant difference in diastolic blood pressure between the two groups ($p < 0.05$).

Comparison of proteinuria that studied in two treatment groups

Variable	Groups		P value*)
	I (n=6)	II (n=6)	
Proteinuria 0	0	1	
Proteinuria +1	0	5	**<0,005**
Proteinuria +4	6	0	
Total	6	6	

Notes
• Group I: Model of Pregnant Mice with Preeclampsia that were not given EPCs CD_{34}

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• Group II: Model of Pregnant Mice with Preeclampsia given that were EPCs CD_{34}

The average proteinuria in the group that given CD34 EPCs was lower than the group that was not given CD34 ECPs (+4 vs +1). Based on Two sample Kolmogorov-Smirnov test, it was found that there was a significant difference in proteinuria in the two groups ($p < 0.05$).

DISCUSSION

This study aims to determine the effect of CD34 endothelial progenitor cells (EPCs CD34) on blood clotting factors and blood pressure in preeclampsia mice. This study used a pure experimental method using 3 stages of research. The first stage was the mating process between male and female mice so that the female mice became pregnant, then they were divided into 2 groups that without and with given the treatment. Both groups were given anti-Qa-2 so that they became models of preeclampsia mice. The second stage was optimization of the isolation culture method pf EPCs CD34 from humans. The third stage was the administration of CD34 EPCs in pregnant mice with preeclampsia that then compared to the controls. The parameters observed were the total of EPCs CD34, the level of platelet aggregation, fibrinogen, D-dimer, PAI-1, blood pressure and proteinuria.

The blood clotting factors that studied were fibrinogen levels, platelet aggregation, PAI-1 and D-dimer. In table 4.1.1, the t test was used for normally distributed data, while the data that were not normally distributed Mann-Whitney test was used. Shapiro Wilk test (for $n < 50$) was used to test data normal distribution.

It was found that the average total of ECPs CD34 in the group that was given EPCs CD34 was higher than in the group that was not given ECPs DCD34 (7.334 ± 0.891 vs 7.040 ± 0.473). Although statistically the difference in the number of EPCs CD34 was not significantly different between the two groups but this is in accordance with several previous studies, for example study by Kwon et al, they calculated the total of EPCs and VEGF levels in the maternal umbilical cord circulation at 31-41 gestational age weeks with preeclampsia ($n = 15$) and compared it with normotensive pregnancies ($n = 30$) in 2007. Kwon et al, found that the number of EPCs and VEGF levels in the umbilical cord blood plasma of women with severe preeclampsia decreased significantly compared to controls.[87]

A study that conducted by Hwang et al which they isolated fetal EPCs from 17 women with preeclampsia (without IUGR) compared to 30 normal pregnant women in 2008. Compared to normal pregnancies, the number of fetal EPCs from women with preeclampsia is very low.[97]

In 2007 Xia et al also conducted a study by collecting umbilical cord vein blood from 14 mothers with preeclampsia and 10 normotensive mothers. It was found that the number of EPCs decreased significantly in the preeclampsia group compared to the normotensive group

Epithelial Progenitor Cells (EPCs) have an important role in the formation of blood vessels and remodeling of endothelial cells in damaged blood vessels. The occurrence of deficiency and impaired function of EPCs in the process of endothelialization of various vascular tissues, including the placenta, it is considered to play a vital role in the underlying causes of endothelial dysfunction in preeclampsia. Failure to mobilize EPCs to the systemic circulation can lead to failure of endothelial cell regeneration, causing disruption of the uteroplacental circulation in

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preeclampsia.[12-15]

The results of this study were different with the hypothesis because there were several limitations in this study. One of them is that this study was a pilot study that tested at the animal level for the first time and there has been no previous research that can provide definite information of EPCs CD34 definite dose and how many times should EPCs CD34 should be given so that it can provide a maximum result to make an increase in the number of EPCs CD34 in the group of preeclamptic pregnant mice. And the number of EPCs CD34 in the group of normal pregnant mice, it is also not known with certainty.

It was found that the average platelet aggregation level in the group that was given EPCs CD34 was lower than the group that was not given ECPs CD34 (27.61 ± 8.93 vs 43.56 ± 1.38). From the t test, it was found that there was significant differences in the platelet aggregation levels between the two groups ($p < 0.05$).

This was consistent with a literature which stated that in preeclampsia, there is an imbalance between prostacyclin and thromboxane. Prostacycline (PGI 2) is synthesized by the endothelium of blood vessels and the renal cortex which has vasodilator properties and inhibits platelet aggregation and can increase uteroplacenta blood circulation. Thromboxane (TXA2) is produced by platelets and has vasoconstrictor characteristic and increases platelet aggregation. In preeclampsia there is a decrease in PGI 2 and an increase in TXA 2, resulting in vasoconstriction and reduced blood flow which inducing platelet aggregation and coagulation factors.[16.17]

Based on research conducted by Harlow et al, platelet activation was only found in cases of preeclampsia, and not in cases of other hypertension in pregnancy. Meanwhile, in a study conducted by Abou-Saleh et al, there was a significant decrease in platelet aggregation level after being given an injection of EPCs (dose dependent) in a mouse model that had previously been induced to experience arterial thrombosis.[98]

These results are also compatible with a study conducted by Alexandru et al, where EPCs were shown to reduce platelet activation and modulate platelet pro-inflammatory and pro-thrombotic agents in a hypertensive-hypercholesterolemic mouse model.[99]

In 2013 Vasilios et al also conducted a study that showed that EPC could reduce platelet activation in vitro, which then it was found that late-outgrowth endothelial cells (OEC) were the most potent types of EPCs in reducing platelet activation.[100]

In 2015 a study by Bou-Khzam et al compared the ability of EPC subtypes to decrease platelet activation, and that were early outgrowth cells (EOCs) and endothelial colony-forming cells (ECFCs). Both have been shown to reduce platelet activation, wherein ECFC is more efficient than EOC in reducing platelet activation.[101]

In Table 4.1.1.3, it was found that the average fibrinogen level in the group that was given EPCs CD34 was lower than in the group that was not given ECPs CD34 (0.944 ± 0.074 vs 1.102 ± 0.065). The t test analysis showed a significant difference in fibrinogen levels between the two groups ($p < 0.05$).

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Fibrinogen plays a role in the interaction between platelets in order for platelet aggregation occurs and also in the stabilization process of platelet aggregation. In pre-eclampsia, there is conversion of fibrinogen to fibrin and circulating in the circulation to form a thrombus in the microvascular and macrovascular, that is why it disrupts blood flow and causes peripheral ischemia and ends with organ damage.[18]

This is consistent with a previous study by Ustun et al in 2005 which stated that fibrinogen and plasma CRP levels in pregnant women with mild preeclampsia and severe preeclampsia were higher than normal pregnant women.[102]

Manten et al 2003 also conducted a study on 14 normal pregnant women and 14 pregnant women with preeclampsia and found higher levels of fibrinogen in pregnant women with preeclampsia. This may be due to excessive inflammatory reactions and endothelial disturbances which are believed to be the key to preeclampsia.[103]

Alwan et al 2013 also found fibrinogen level was higher in 35 pregnant women with severe preeclampsia than in 35 normal pregnant women.[104]

It was found that the average D-Dimer level was higher in the group that was given EPCs CD34 than the group that was not given ECPs CD34 (7,778 vs 2,266). It was found that there was significant differences in D-Dimer level between the two groups ($p < 0.05$) from Mann-Whitney test.

D-dimers is the result of cross-linked fibrin degradation, in which fibrinogen is converted into fibrin monomers and polymerizes into fibrin polymers, it is activated by factor XIIIA and it is a protein that released into the circulation during the fibrin breakdown process. D-dimers can be used as an indicator of thrombus occurrence that will be breakdown somewhere in the body. In severe preeclampsia, D-dimers can be used as an initial screening and follow-up test for coagulopathy and to identify progression of more severe disease. A positive D-dimer means that there is a high level of fibrin degradation products in the body and this indicates a significant amount of thrombus. Endothelial dysfunction can cause the non-thrombogenic surface to turn thrombogenic, so that coagulation activation can occur. One way to determine the presence of thrombosis in the blood vessel circulation is to measure the D-dimer level.[16,17]

According to Bardbury et al, in their 2019 study regarding the relationship of high EPCs levels with the risk of recurring deep vein thrombosis (DVT), it was stated that the increased D-dimer level after EPC administration might be due to the not optimal dose of EPCs, so further research is needed to find the optimal dose of EPCs to reduce D-Dimer levels.[105]

It was found that the average PAI-1 levels in the group that was given EPCs CD34 was higher than those that was not given ECPs CD34 (4.168 ± 0.564 vs 3.565 ± 0.802). However, there was no significant difference in CD34 EPCs levels between the two groups ($p > 0.05$) from t test.

Plasminogen activator inhibitor-1 (PAI-1) is a protease inhibitor of tissue plasminogen activator, and is an important inhibitor of fibrinolytic activity. Based on the literature, PAI-1 plays an important role in the process of preeclampsia and HELLP syndrome, which there is an excessive release of PAI-1 from the endothelium and cause thrombosis in the spiral or intervillus arteries

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which then results in decreased placental perfusion. PAI-1 can be found on the extravillalintersisialtrophoblasts and the vascular trophoblasts of human placenta. During the implantation and placentation processes, PAI-1 is responsible for inhibiting the degradation of the extracellular matrix, which inhibits trophoblast invasion and results in failure of spiral artery remodeling and thus endothelial dysfunction.[2,19.92]

This theory is supported by a study conducted by Ye et al that stated, there is a significant increase in PAI-1 in preeclampsia patients when compared to pregnant women who do not have hypertension.[106]

In 2013 Morgan et al stated that the fibrinolysis pathway also play a role in the pathogenesis preeclampsia is regulated by the PAI-1 gene. A research conducted by Udenze et al in 2017 also found that PAI-1 level was higher in pregnant women with preeclampsia compared to normal pregnant women.[107,108]

However, the results of this study do not support previous research, according to study in 2007 by Smadja et al, because apart from having a role in the angiogenesis process endothelial progenitor cells can also have anticoagulant and antifibrinolytic effects, and Smadja et al found an increase in PAI-1 level up to 10 times.[109]

In Tables 4.1.1.6 and 4.1.1.7 the mean systolic and diastolic blood pressure in the group that was given EPCs CD34 was lower than the group that was not given ECPs CD34. There was a significant difference in systolic blood pressure between the two groups ($p < 0.05$) from t test analysis.

Preeclampsia is a condition which there is an increase in systolic blood pressure ≥140 mmHg and diastolic blood pressure ≥90 mmHg in pregnancies over 20 weeks with proteinuria ≥ 300 mg in 24-hour urine or ≥ 1 + dipstick. In preeclampsia there is a balance issue between thromboxane (TXA 2) which is a strong vasoconstrictor and prostacycline (PGI 2) which is a strong vasodilator. TXA 2 levels that are higher than PGE 2 will cause symptoms of hypertension in pregnancy. Decreasing blood pressure is one of the therapeutic targets in the management of preeclampsia cases.[1,21,50]

According to Berezin in 2018, there is a relationship between hypertension and a decrease number of EPCs. Based on that, EPCs plays an important role in maintaining vascular integrity, preventing changes in the endothelium and preventing endothelial dysfunction.110

Another study in 2010 by Fadini et al, in the preliminary clinical trial the used of EPCs in patients with pulmonary hypertension has a beneficial effect, so the use of EPCs as therapy in pulmonary hypertension can be considered.[111]

It was found that the average proteinuria in the group given that was EPCs CD34 was lower than the group that was not given CD34 ECPs (+4 vs +1). From the Two sample Kolmogorov-Smirnov test, it was found that there was a significant difference in proteniuria between the two groups ($p < 0.05$).

Preeclampsia is a condition where there is an increase in systolic blood pressure ≥140 mmHg

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and diastolic blood pressure ≥90 mmHg at 20 weeks gestation accompanied by proteinuria ≥ 300 mg in 24-hour urine or ≥ 1 + dipstick.[1,21]

Urine protein examination that can be performed on pregnant women is one type of laboratory examination to determine kidney function during pregnancy and identify the presence of preeclampsia. Proteinuria detection is very important in the diagnosis and management of hypertension in pregnancy. Proteinuria occurs because there is lesion in the glomerulus. Proteinuria is the last symptom to appear in preeclampsia patients.[1,21]

The administration of EPCs CD34 to pregnant rats with preeclampsia was proven to show clinical improvement, which was marked by a decrease in proteinuria levels. This result is supported by a study in 2018 by Ozkok et al which stated that EPCs play a role in treating heart disease and kidney disease. Moreover study in 2016 by Kiewisz et al stated that EPCs play a role in improving heart disease and kidney disease.[112,113]

Conclusion

There is no difference in the number of EPCs CD34 and PAI-1 in the group of preeclamptic pregnant mice that was given EPCs CD34 compared to the group of preeclamptic pregnant mice who was not given EPCs CD34. Platelet aggregation levels, fibrinogen levels, blood pressure and proteinuria in the preeclamptic pregnant mice group who was given EPCs CD34 were lower than the preeclamptic pregnant mice group that was not given EPCs CD34. D-dimer level in the preeclamptic pregnant mice group who was given EPCs CD34 was higher than the preeclamptic pregnant mice group that was not given EPCs CD34.

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