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Expression of Foxp3 mRNA on Preeclampsia with Adaptation Theory

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ABSTRACT

This study aims to determine the expression of mRNA Foxp3 on Preeclampsia and normal pregnant .The research method used is through the quantitative research method using case-control design with the sample size of 20 respondents consisting of 10 cases and 10 controls. The case samples were pregnant women with preeclampsia at the time of the study while control samples were normal pregnant women who met inclusion and exclusion criteria. The data was analyzed using Independent T-Test statistics.

The results showed that 95% CI with LL = 5,563 and UL = 7,219 with p = 0,000 mean that average expression of Foxp3 mRNA in expressed blood was lower in the preeclampsia group than the normal pregnant group. While 95% CI with LL = 5,319 and UL = 7.047, with p-value = 0.000 means that the average expression of Foxp3 mRNA expressed in the placenta is also lower in the preeclampsia group than the normal pregnant.

Keywords: *Foxp3 mRNA expression, Preeclampsia,*

INTRODUCTION

Preeclampsia is a complication of pregnancy with a worldwide incidence of 2-10%.It is also one of the leading causes of maternal and perinatal morbidity and mortality.[1]

Included in the ASEAN countries, Indonesia has the highest mortality rate of 330/100 and a perinatal mortality rate of 420 / 100,000 live births. Maternal mortality varies in different regions with a range of 330 - 700 / 100,000. Perinatal mortality rates can be dropped drastically, but maternal mortality has not shown any sign of decrease.[2]

Causes of maternal and perinatal mortality: immediate causes of complications of pregnancy and childbirth such as bleeding 60-70%, preeclampsia and eclampsia 10-20%, infection 10-20% including partus are abandoned, others: amniotic fluid embolism and anesthesia. Underlying causes of indirect mortality such as lack of nutritional status, delay providing adequate help[2,3,4]. Preeclampsia plays a role in intrauterine death and perinatal mortality. The main cause of neonatal death due to preeclampsia is placentalin sufficiency and placenta solution. Retardation of growth in the womb is also common in infants whose mothers suffer from preeclampsia. Human epidemiological data suggest that children born to mothers of preeclampsia are at risk for long-term neuro developmental development.[5]

Regulatory T cells have an important role in immune homeostasis. One of the concerns in various studies in both infectious and non-infectious diseasesis the role of regulatory T cells with Foxp3 as its transcription factor. Thestable Foxp 3 expression is indispensable for the development of T regulatorycellfunction.[6]

Foxp3 findings provide a large role in identifying regulatory T cells. The Forkhead P3 (Foxp3) gene is the master control gene for the development and function of the T regulator which plays an important role in the maintenance of self-tolerance and mediates maternal tolerance to the fetus. The lack of regulatory T cells (T reg) or functional deficiencies associated with infertility, miscarriage, and preeclampsia.[7,8]

Signs and symptoms occur only during pregnancy and disappear quickly after the fetus is born. No particular profile identifies women who will suffer from

preeclampsia. However, there are certain risk factors associated with disease progression: primigravida, multigravida, large fetus, multiple fetal pregnancies, and morbid obesity. Preeclampsia is an inseparable disease of mild to severe preeclampsia, HELLP syndrome or eclampsia.[9]This study aims to determine the expression of mRNA Foxp3 on Preeclampsia and normotension.

MATERIALS AND METHOD

This research was conducted at BLUD RSUD H. Padjonga Daeng Ngalle, Takalar District, from October 2016 to May 2017. The population of this research is all pregnant women preeclampsia and normotension. The samples in this study are pregnant women who meet the inclusion criteria of primigravida and multigravida with a third-trimester pregnancy age. The sampling technique used was the purposive sampling method consisting of 20 samples of patients with two groups: 10 samples of pregnant women with preeclampsia and 10 samples of pregnant women with normotension. In this study interviews were conducted to obtain information about the characteristics and general circumstances of the subjects such as name, age, number of children, history of previous illness, and health service history by looking at KIA books/midwife notebook, preeclampsia with blood pressure criteria ≥ 140 / 90mmHg as cases and blood pressure less than 140/90 mmHg as control. Intake of a urine sample for proteinuria examination. Examination of edema in sacrum area above the tibia, wrist, and feet. Furthermore, blood sampling of respondents was taken for examination of their mRNA gene Foxp3 expression. Specimens of blood research subjects were taken when the mother visited the ANC room to mother the childbirth and then taken the placenta tissue subsequently carried out an examination of immunology and molecular biology laboratory Faculty of Medicine, Hasanuddin University , Makassar ,Indonesia using the molecular technique that is Real-Time Polymerase Chain Reaction (RT-PCR). Data analysis with Independent T-Test were tested using SPSS version 20 statistic program.

RESULTS

Table 1 shows that the age of respondents in the normotension group were from <20 and >35 years of age as many as 2 respondents, while the age of 20-35 years were 8 respondents.

Table 1: Characteristics of Respondents on Preeclampsia and Normal pregnant

Variables	Normal pregnant		Pre-eclampsia	
	n	(%)	n	(%)
Age				
< 20 and> 35 year	2	20	5	50
20–35	8	80	5	50
Parity				
1–3	7	70	100	100
> 3	3	30	0	0

While the age of respondents in the preeclampsia group was <20 and >35 years old as many as 5 respondents, while the age of 20-35 years as many as 5 respondents. While based on parity shows that the highest percentage are respondents with 1-3 persons in the group preeclampsia group at 70% while respondents with parity greater than 3 people in the case group at 30%. While in the group of normal pregnant parity was 1-3 people as much as 10 or 100%.

Table 2: Expression of mRNA Foxp3 expression in blood

Expression *mRNA Foxp3* blood (LL-UL)	n	mean	SD	95% CI	*p* value
Normal pregnant	10	12,123	0,967	(5,563-7,219)	0,000
Preeclampsia	10	5,732	0,779		

Table 2 shows that the average expression of blood binding mRNA in the normal pregnant group was 12.123 Ct with a standard deviation of 0.967 Ct, whereas in preeclampsia was 0.5723 Ct with a standard deviation of 0.779 Ct. Based on statistical analysis results showed LL = 5,567 and UL = 7,216 with a value of p = 0.000 means that the average expression of the Foxp3 mRNA in the expressed blood is higher in normal pregnant group and lower in preeclampsia group.

Independent T-Test

Table 3: Expression of Foxp3 mRNA expression in the placenta

Expression *mRNA Foxp3* placenta (LL-UL)	n	mean	SD	95% CI	*p* value
Normal pregnant	10	12,597	1,027	(5,319-7,047)	0,000
Preeclampsia	10	6,413	0,798		

Independent T- Test : Table 3 shows that the average expression of Foxp 3 mRNA on the placenta of the normotensive group was 12.597 Ct with a standard deviation of 1.027 Ct, whereas in preeclampsia was 6.413 Ct with a standard deviation of 0. 798 Ct. Based on statistical test results, 95% CI with LL=5, 319 and UL=7.047 with p=0,000 means that the average expression of Foxp 3 mRNA on the placenta is expressed higher in the normotensive group and expressed lower in preeclampsia group.

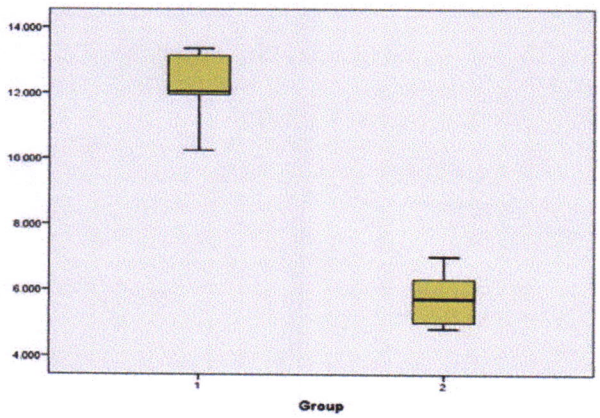

Figure 1: Foxp3 mRNA expression in blood normal pregnant (group 1) Preeclampsia (group 2)

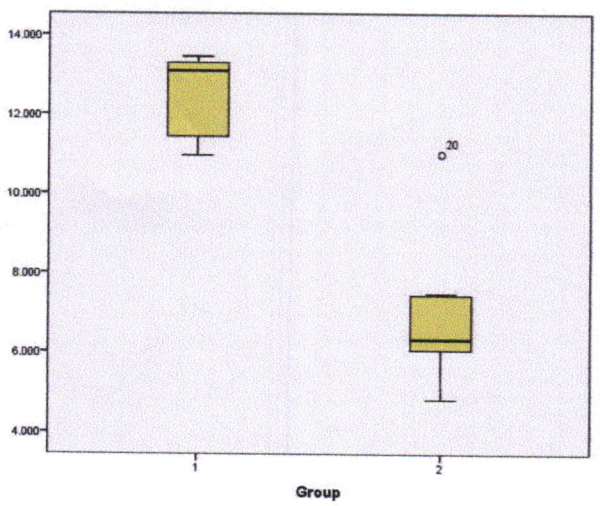

Figure 2: Foxp3 mRNA expression in placenta normal pregnant (group 1) Preeclampsia (group 2)

DISCUSSION

Preeclampsia is a special condition in pregnant women with maternal and infant effects characterized by hypertension and proteinuria with or without edema appearing in pregnancies over 20 weeks.

Pregnant women tend to be susceptible to preeclampsia if they have the following predisposing factors: nullipara, age <20 or> 35 years, multiple pregnancies, family history with preeclampsia, preeclampsia history, previous eclampsia, renal disease, hypertension, and diabetes mellitus that existed before pregnancy and obesity.

This research generally aims to find out the expression of mRNA Foxp3 on preeclampsia and normal pregnant, then from the research result is discussed as follows:

The Foxp3 gene is a member of the forkhead/winged helix family of tractional regulators whose results are dysfunctional in T-cell hyperactivation. Located on the X chromosome (Xq11.23 - Xq13.3) consists of 11 exons and that encodes a 43α molecule (431a) protein with a molecular weight of 47, 25 kD. In healthy conditions, Foxp3 Treg can play a role in the prevention and treatment of diseases associated with self-tolerance disorders because of its function as a transcription factor regulator T cells that play an important role in peripheral tolerance. Foxp3 expression is lower in patients who are positive for rheumatoid arthritis compared to healthy people and DNA methylation is a factor that is closely related to the expression of Foxp3[11]. Children diagnosed with type 1 diabetes have decreased Foxp3 induction.[12]

Statistical results showed that Foxp3 mRNA expression was significantly lower in blood in the preeclampsia group and expressed higher in normal pregnant group ($p < 0.05$). This is in line with the results of research conducted by Chen, at al.[13]. Foxp3 expression was significantly lower than control ($p<0.05$). Treg is significantly reduced in the blood of preeclampsia compared with healthy women[14] .Foxp3 Treg has a lower percentage of peripheral blood in the third trimester of preeclampsia compared to normal pregnant women[15,16]. Foxp3 mRNA expression levels declined both in peripheral blood mononuclear cells and decidua from patients with preeclampsia compared with healthy pregnant women ($p < 0.05$)[17].

The age of respondents in this study was <20 years and >35 years. This is consistent with the study of Asmana,et.,al[18], predominantly severe preeclampsia occurring in the <20 and >35 years age group. At <20 years of age, uterine size has not reached the normal size for pregnancy while age >35 years is a degenerative process

that results in structural and functional changes that occur in the blood vessels. Endothelial dysfunction is an increase in nitric oxide, vascular cell adhesion molecule-1 (VCAM-), ICAM-1, Prostacyclin (PGI2). In this condition, there is an imbalance of vasoactive substance so that hypertension can occur. Endothelial dysfunction also causes increased vascular permeability resulting in edema and proteinuria.Cell-mediated immunity produces T-helper cells Th1 and Th2 which will play a role in the activity of macrophage cells to activate NK cells with cytokines in the process of pregnancy[20]. T-cell regulator with Foxp3 transcription factor is considered to inhibit T-helper 1 (Th1) and T-helper 2 (Th2) cells. The opinion that T regulators prevent the development of Th1 and Th2 through direct contact[21].

Continuous expression of Foxp3 is needed to maintain transcription and program function during the development of Treg cells[22]. The balance of the immune system and the functioning of the autoimmune response is the result of homeostasis between T cell effectors with T regulator cell activity. The induction of the Foxp3 gene in normal naïve T cells converts it into regulatory T cells. Direct T cell proliferation is usually followed by decreased or even dysfunctional Treg cell function so that the therapeutic agent that receives much attention is that it can affect both the effectors of T cells as well as on T regulatory cells. Treg cells are associated with preeclampsia. Foxp3 is involved in the development and functionality of Treg[23]. Immune suppression is a major function of regulatory T cells (Treg) or Th3 which act to limit the specific antigenic immune response[24].The decrease of Foxp3 and T regulator as the body response is maladaptive namely preeclampsia. In line with Lockwood's research, 2008[20], deviation of adaptation to the immune system will lead to a mal-adaptation of the maternal immune system that will clinically cause preeclampsia.

Conversely, if the body's response is adapted so that no endothelial dysfunction occurs, the Foxp3 expression is elevated to maintain immune system balance as macrophage activation and pro-inflammatory cytokine increase as a normal response of the body. T increased regulator that transcribed Foxp3 needed to control the balance of immune response in order to avoid excessive response and result in tissue damage so that pregnant women do not experience preeclampsia. Inadequate expression of Foxp3 is associated with recurrent spontaneous abortion, infertility, repetitive implantation failure and preeclampsia. Normal pregnancy requires mother's immunity as tolerance to the fetus. During pregnancy, the immune system should maintain tolerance to the fetus, so changes in cytokine balance can cause the pregnancy to be impaired. Treg cells play an important role in maintaining the immune system.[8,14].

Based on statistical test results showed 95% CI value with LL value = -1,422 and UL=0,0599 with p-value =0,490 (p>0,05) meaning that there is difference of expression of mRNA Foxp3 of blood and placenta in group of preeclampsia that is not significant. The results of this study are in line with a study conducted by Prins,et.,al.[25] in pregnant women with preeclampsia having a percentage of Foxp3 and T regulators significantly lower in blood.

Endothelial dysfunction is caused by exposure to inflammatory cytokines, oxidative stress, and hypercholesterolemia. As a result of endothelial dysfunction occurs placental blood perfusion. Babies born to preeclamptic mothers will have neonatal asphyxia, this is because in preeclampsia there is a decrease in placental blood supply and result in the baby experiencing hypoxia. Prematurity is associated with high placental cortisol in preeclampsia (OR 0.12,95% CI 0.02- 0.5)[26].

Preeclampsia is a systemic disease of maternal endothelial dysfunction and immune maladaptation in the placental compartment caused by inadequate induction of maternal tolerance to paternal antigen and unfavorable combinations of genetically maternal leukocyte receptors and fetal antigen[27].

CONCLUSION

Foxp3 mRNA expression is lower in the blood in preeclampsia than in normal pregnant. Foxp3 mRNA expression is lower in placenta than normal pregnant.

Conflict of Interest: No obstacles were found in the sampling of blood and placenta at the hospital in both the preeclampsia and normotensive patients

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Ethical Clearance: The Ethical Clearance obtained from the Hasanuddin University committee with number UH16080648

21. Orihara ,K.,Nakae S, Pawankar,R., and Saito. H,. Role of Regulatory and Proinflammatory T-Cell Populations in Allergic Diseases. WAO, Journal 2008,9-14

22. Wiiliams L.M, Rudensky A.Y. Maintenance of the Foxp3–dependent developmental program in mature regulatory T cells requires continoud expression on Foxp3 . Nat Immunol 2007; 8 : 277-84.

23. Nourouzian M, 2016. Foxp3 Gene Promoter Polymorphism Affects Susceptibility to Preeklampsia Hum Immunol

24. Park O, Grishina I, Leung PS, Gershwin ME, Prindiville T. Analysisof the Foxp3/Scurfin gene in Crohn's disease. Ann N Y AcadSci2005;1051:218–28.

25. Prins,J.R.,Boelens.H.MHeimweg,J.,van der Heide ,Dubois, A.E., van Oosterhout., 2009. Preeclampsia is Associated with Lower Percentasges of Regulatory T Cells in Maternal Blood., Journal Hipertension of Pregnancy.

26. Aufdenblatten, M .,Baumann M, RaioL.,Dick B., Frey BM., Schneider H.,Surbek D., Hocher B., Mohaupt MC.,2009. Prematurity is related to high placental cortisol in preeclampsia. Pubmed

27. Cudihy .D & Lee,R.V.,2009. The Pathophysiology of Preeclampsia : Current Clinical Concepts. Journal of Obstetrics and Gynaecology. Vol.29. Pages 576-582.