INTRODUCTION

Preeclampsia (PE) is the most common disorder in pregnancy and is one of the leading causes of maternal and fetal morbidity and mortality. PE affects 1-10% of pregnant women worldwide and is characterized by high blood pressure and proteinuria that occur after 20 weeks' gestation.\(^1\) The etiology of PE is not yet fully understood, but there is a theory that says the placenta is closely related to the pathogenesis of PE. This relates to disorders between blood vessels and anti-angiogenic responses.\(^2\) Another hypothesis explains that the etiology of PE is related to the immune response. Cytokines are substances that may be involved in the pathogenesis of PE. Type 1 cytokine receptors (interleukin-2), and type 2 cytokine receptors (interferon and alpha necrosis tumours) are generally produced in inflammatory processes caused by PE. Some previous studies have even shown variations in cytokine levels in women with PE even though the pathogenesis in this disease is still controversial.\(^3\) Cytokines are divided into 6 groups: interleukin, interferon, Colony-Stimulating Factor, Tumor Necrosis Factor,
Growth Factor, and Chemokine. Several types of cytokines have been shown to increase in preeclampsia and may be used as a marker of the diagnosis of preeclampsia. Inflammatory cytokines, such as IL-6 and TNF-α, are reported to be increased in preeclampsia.\textsuperscript{4} Tumour Necrosis Factor-α (TNF-α) is a proinflammatory cytokine that can exert various effects on various cells.\textsuperscript{5} Hypoxia triggers the production of TNF-α which has a cytotoxic effect on endothelial cell blood vessels, placental trophoblast cells. This induces apoptosis and inhibits trophoblast cell proliferation, which causes vasocostriction. TNF-α levels in the maternal circulation increase before the clinical manifestations of preeclampsia.\textsuperscript{6} A study conducted in 2019 showed TNF-α levels were significantly increased (p <0.05) in women with preeclampsia compared to women with normal pregnancies.\textsuperscript{1}

Another risk factor commonly associated with PE is ethnicity. In 2012, some studies showed Turkish and Moroccan women had a lower risk of PE compared to women from ethnic Dutch.\textsuperscript{7} Another study in 2016 showed that black women had a higher percentage of PE development compared to white women.\textsuperscript{8}

This study was conducted to analyze differences in TNF-α levels in pregnant women with PE in the Bugis, Makassar, Mandarin, and Toraja tribes as a prognosis and prevention efforts for PE.

**MATERIALS AND METHODS**

**Research sites**

This research was conducted in March-July 2020 and has received an ethical approval recommendation with protocol number UH20020139. This research was conducted at the Hasanuddin University Hospital, Health center Jumpandang Baru, Health center Barabaraya, Health center Makale, Elim Toraja Utara hospital, Lakipada Tana Toraja Regional Hospital, Totoli Health Center, Majene Public Hospital, Mattirobulu Health Center, and Lasirrang Pinrang General Hospital.

**Data types and sources**

Data collected from respondents are demographic data (age, BMI before pregnancy, and history of PE). Data were taken from pregnant women of gestational age > 20 weeks with a diagnosis of PE without comorbidities.

**Data collection technique**

Demographic data were collected using a questionnaire through the method of direct interviews with respondents.

Meanwhile, for blood sampling, researchers were assisted by laboratory personnel at the Health center and research hospitals. The collected samples were then centrifuged and stored in the refrigerator at \(-20^{\circ}C\). After all the samples were met, TNF-α levels were examined using the Human TNF-α ELISA Kit, Bioassay Technology Laboratory at Hasanuddin University Medical Research Center (HUM-RC) Laboratory. Data were analyzed using the Chi-Square test, the Shapiro-Wilk test, the Kruskal-Wallis test, and the Mann-Whitney test.

**RESULTS**

Data shows that three characteristics, age, parity, and BMI among cases and control groups are almost similar. It’s just that the characteristics of BMI in the ‘normal’ category, data shows total respondents of the control group highest than the case group (Table 1).

The results of the analysis showed that differences in TNF-α levels between PE pregnant women and normotension significant in Makassar and Toraja tribes. The mean rank of PE pregnant women in the Makassar tribe was 16.82, lower than the normotensive pregnant women 6.18 (p <0.001). Similarly, the Toraja tribes, the mean rank of PE pregnant women was 14.55, also lower than the normotensive pregnant women 8.45 (p = 0.028). On the other hand, Bugis and Mandarin tribes also show differences in TNF-α levels of PE pregnant women with normotensive pregnant women, but not as significant as the Makassar and Toraja tribes (Table 2).

| Table 1: Demographic Characteristics of Respondents |
| --- |
| Characteristics | Preeclampsia (n = 44) | Normal (n = 44) | p-value |
| Age n (%) | | | |
| High risk | 7 (46.7) | 8 (53.3) | 0.500 * |
| Low risk | 37 (50.7) | 36 (49.3) | |
| Parity n (%) | | | |
| Primigravida | 14 (51.9) | 13 (48.1) | 0.500 * |
| Multigravida | 30 (49.2) | 31 (50.8) | |
| BMI n (%) | | | |
| Underweight | 0 (0) | 1 (100) | |
| Normal | 8 (30.8) | 18 (69.2) | 0.005 * |
| Overweight | 21 (48.8) | 22 (51.2) | |
| Obesity | 15 (83.5) | 3 (16.7) | |

*chi-square test
Table 2: Differences TNF-α levels between preeclampsia pregnant women and normotensive pregnant women in the Bugis, Makassar, Mandar, and Toraja tribes (n = 88).

| Tribes   | Preeclampsia Status | TNF-α Levels | p-value* |
|----------|---------------------|--------------|----------|
|          | Mean rank           | Sum of rank (Pg/ml) |          |
| Makassar | Preeclampsia        | 16.82        | 185.00   | <0.001  |
|          | Normotension        | 6.18         | 68.00    |         |
| Bugis    | Preeclampsia        | 12.64        | 139.00   | 0.438   |
|          | Normotension        | 10.36        | 114.00   |         |
| Mandar   | Preeclampsia        | 13.48        | 145.00   | 0.243   |
|          | Normotension        | 9.82         | 108.00   |         |
| Toraja   | Preeclampsia        | 14.55        | 160.00   | 0.028   |
|          | Normotension        | 8.45         | 93.00    |         |

*u: Mann-Whitney test

DISCUSSION

Based on statistical tests in table 1 shows that age and parity did not significantly influence the incidence of PE. Meanwhile, the test results for BMI showed a value of p = 0.005 which means BMI influences the incidence of PE, there were 43 overweight people (48.9%), and 18 obese people (20.5%). A strong correlation was found between an increase in BMI and the risk of preeclampsia. The risk increased twofold for mothers with a BMI category > 25 kg/m², and almost three times for mothers with a BMI category > 30 kg/m². The increased risk affects not only white and African American women but also women of other ethnicities around the world.9 Someone in the obese category shows metabolic syndrome which is characterized by insulin resistance with an excessive flux of fatty acids that contribute to disturbing the immunological balance.10 On the other hand, metabolic syndrome is associated with endothelial dysfunction. Theory suggests that endothelial dysfunction, increased vascular permeability, and increased levels of circulating endothelial cell markers play an important role in the pathogenesis of preeclampsia.11

The results of the statistical test showed that from the four tribes, Makassar and Toraja ethnic groups had a significant difference in levels of TNF-α, with p-values <0.001 and 0.028, respectively. The results of this study are in line with the previous study which showed preeclamptic pregnant women had a high pro-inflammatory cytokine response, while the anti-inflammatory response decreased. The higher levels of proinflammatory cytokines in the PE group were TNF-α and IL-15, while IL-10 had lower levels.7 There is also a study that shows the same thing, that TNF-α levels were found to be significantly increased in preeclamptic pregnant women compared to normotensive pregnant women (p = 0.04).12 Another study that is in line with the results of this study is a study conducted by Szarka et al. which showed TNF-α was significantly different in PE pregnant women compared to normotensive pregnant women (p <0.001).13 TNF-α is a pro-inflammatory cytokine that plays a role in regulating many aspects of macrophage function. TNF-α is released immediately in conditions of trauma, infection, or exposure to bacteria. This proinflammatory cytokine has proven to be one of the most numerous early mediators in the inflamed tissue and is considered a “master regulator” of pro-inflammatory cytokine production.14 TNF-α plays an important role in the pathogenesis of preeclampsia. There is the fact that higher circulating TNF-alpha levels are observed in preeclampsia than in gestational hypertension, suggesting an association with disease severity.12 Inflammation is part of the complex biological response of the vascular network to harmful stimuli. The progressive destruction of tissue will endanger the survival of the organism. The inflammatory response is usually accompanied by an increase in the concentration of proinflammatory cytokines, an acute-phase protein, and may involve endothelial activation. Hypoxia promotes the overproduction of TNF-α, a proinflammatory cytokine factor, and has a potent cytotoxic effect on vascular endothelial cells, placental trophoblast cells. It induces apoptosis, inhibits trophoblast cell proliferation, increases endothelial expression of platelet-derived growth factor, endothelin-1, and plasminogen-1 activator inhibitor, all of which are associated with vasoconstriction.6 Besides, a poorly perfused and hypoxic placenta is thought to synthesize an increased vasoactive factor, namely TNF-alpha. This increase can lead to endothelial dysfunction by reducing bioavailable nitric oxide and increasing reactive oxygen species and endothelin-1 which in turn leads to altered renal function, decreased renal pressure natriuresis, increased total peripheral resistance, and hypertension. The ischemic placenta leads to endothelial cell activation/dysfunction and increased TNF-alpha synthesis which leads to induced structural and functional changes in endothelial cells leading to preeclampsia through TNF-alpha regulation of endothelin-1 gene transcription.12

The difference in TNF-α levels is influenced by several factors, severe infections can trigger the production of large amounts of TNF which cause systemic reactions, age, and ethnicity, infectious diseases, autoimmune, obesity, habit, smoking and alcohol consumption, high activity levels, history of oral contraceptives, parity, and advanced age at first pregnancy.15-17 In this study, it was found that from four tribes, Makassar and Toraja had significant differences in levels of TNF-α levels of pregnant women with PE and normotension. Makassar and Toraja are regencies in South Sulawesi with different geographical locations, as well as air temperatures and sample characteristics. The geographical conditions of Makassar City, based on the altitude, vary between 0-25 meters above sea level, with an air temperature of 20°C-32°C. While Tana Toraja Regency is a plateau with
altitudes ranging from 300 -> 2,889 meters above sea level. The temperature in Tana Toraja ranges from 16° - 28°C. A study conducted by Palmer et al. showed that in general, the incidence of preeclampsia was more common in the highlands (± 3100m) (p <0.002). Highland dwellings increase the risk of experiencing preeclampsia 3.6 times. There is also research conducted by Coussons-Read et al. showing that TNF-α levels were found to be significantly increased in pregnant women who live in the highlands compared to pregnant women who live in the lowlands (p<0.045). This is related to the theory that pregnant women who live in the highlands are more prone to placental hypoxia caused by a lack of oxygen supply, which in turn causes oxidative stress and endothelial dysfunction, vasoconstriction occurs and leads to increased blood pressure.

The difference in terms of air temperature in Makassar and Toraja was also discussed by Shahgheibi et al. in their research which concluded that the incidence of preeclampsia was higher in winter than in summer. Temperature changes affect plasma volume, in cold weather can cause vasoconstriction that can trigger the development of pathogenesis from preeclampsia. Another relationship is explained Madhu et al. in their research that the risk of preeclampsia increases in winter (p<0.001) due to vitamin D deficiency. Kasitriol (the active form of vitamin D) functions as a hormone that regulates gene function in the implantation process. Vitamin deficiency can lead to abnormal implantations that trigger an inflammatory response, which is the precursor to the development of preeclampsia.

**CONCLUSION**

This study concluded that there are differences in TNF-α levels in preeclampsia pregnant women and normotensive in the Makassar and Toraja tribes. So that tribal variation can be considered as a risk factor for preeclampsia pregnant women.

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