https://doi.org/ 10.33472/AFJBS.6.1.2024.171-183

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

The Alteration Of TNF-A, MMP-9, And E-Selectin Expression In BALB/C Mice As A Model Of Pregnancy-Induced Hypertension Induced By Salted Fish Extract And NaCl

Novi Anggraeni[ae], Agus Sulistyono [b]*, Gwenny Ichsan Prabowo[c], Aditiawarman[d]

[a]Doctoral Program of Medical Science, Faculty of Medicine, Universitas Airlangga University, Jl. Prof. DR. Moestopo No.47, Pacar Kembang, Kec. Tambaksari, Surabaya, Jawa Timur 60132, Indonesia.

[b]Departement of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Jl. Prof. DR. Moestopo No.47, Pacar Kembang, Kec. Tambaksari, Surabaya, Jawa Timur 60132, Indonesia,

[c]Departement of Physiology and Medical Biochemistry, Faculty of Medicine Airlangga University, Jalan Dr. Ir. H. Soekarno No. 123, Mulyorejo, Kota Surabaya, Jawa Timur 60115, Indonesia

[d]Departement of Obstetrics and Gynecology, Faculty of Medicine, Airlangga University, Jl. Prof. DR. Moestopo No.47, Pacar Kembang, Kec. Tambaksari, Surabaya, Jawa Timur 60132, Indonesia

[e]Institute of Health Science of Ngudia Husada Madura, Jl RE. Martadinata No.45, Wr 06, Mlajah, Kec. Bangkalan, Kabupaten Bangkalan, Jawa Timur 69116

*Corresponding author Fax number: +6231 5020251; Tel.: + 6282131413160

*E-mail: agus.sulistyono@fk.unair.ac.id

Article History
Volume 6,Issue 1, 2024
Received:21 Mar 2024
Accepted : 22 Apr 2024
doi: 10.33472/AFJBS.6.9.2024.171-183

ABSTRACT

Context: Pregnancy-induced hypertension is a unique condition characterized by elevated blood pressure and proteinuria that typically resolves after delivery. It affects approximately 5 to 8% of pregnancies. Pregnancy carries the risk of increased proinflammatory cytokines such as TNF-α, IFN-γ, IL-6, and IL-8, which can contribute to an inflammatory response through NF-kB activation, and changes in matrix metallopeptidase gene polymorphism via placental ischemia processes. E-selectin concentration has been reported to increase in patients with chronic hypertension due to endothelial dysfunction. MMP-9 is constitutively present in neutrophils, where an imbalanced level of MMP-9 can disrupt physiological trophoblast invasion processes.

Objective: To demonstrate changes in the expression of MMP-9, TNF-α, and E-selectin exposed to salted fish extract and NaCl as a model of pregnancy-induced hypertension in BALB/c mice.

Method: True experimental with a Post Test only with control group design. The research subjects were BALB/c mice at 1-day gestation age impregnated using the estrus synchronization technique. Data analysis was conducted using the non-parametric KruskalWalls test and parametric one-way ANOVA with SPSS 22 software.

Results: There was a significant difference between the control group and the treatment group in the expression of TNF-α, E-selectin, and MMP-9 (p=0.000).

Conclusion: There is a significant difference in the expression changes of TNF-α, E-selectin, and MMP-9 following the administration of salted fish extract and NaCl in BALB/c mice as a model of pregnancy-induced hypertension.

Keywords: Extract, Salted Fish, NaCl, Hypertension, Pregnancy, TNF-α, E-selectin, MMP-9.

INTRODUCTION

Hypertension during pregnancy affects both the mother and the fetus, it can lead to maternal and fetal morbidity and mortality if not properly managed [1]. Preeclampsia is a pregnancy-related hypertensive disorder characterized by placental malperfusion and multi-organ injury. Hypertension is a risk factor for the occurrence of preeclampsia; in other words, a history of hypertension increases the risk of preeclampsia by 1.591 times compared to those without a history of hypertension [2]. Pregnancy-induced hypertension is a unique condition characterized by symptoms such as elevated blood pressure and proteinuria, which typically resolve after delivery [3]. Approximately 5-8% of pregnancies may experience hypertension, manifesting in one of the following categories: chronic hypertension, preeclampsia-eclampsia, or chronic hypertension with superimposed preeclampsia [4]. Pregnancy carries the risk of increased proinflammatory cytokines and changes in matrix metallopeptidase gene polymorphism through placental ischemia processes [5]

Based on data from the World Health Organization (WHO) in 2020, an estimated 934 cases of preeclampsia occur worldwide each day. Approximately 342,000 pregnant women experience preeclampsia, with 25% of preeclampsia/eclampsia being the leading cause of complications during pregnancy and childbirth [6]. Preeclampsia causes 9-26% of maternal deaths in developing countries and 16% in developed countries [7]. This condition accounts for about 14% of maternal deaths and 10-25% of perinatal deaths globally. Additionally, preeclampsia also increases the risk of chronic diseases later in life for both mothers and children, such as hyperthyroidism, diabetes mellitus, and dyslipidemia [8]. Hypertension (eclampsia-pre-eclampsia) accounts for 33.07% of the highest causes of death, followed by hemorrhage at 27.03%, non-obstetric complications at 15.07%, obstetric complications at 12.7%, infections during pregnancy at 6.06%, and other causes at 4.81% [9]. The prevalence of pregnancy-induced hypertension increased from 10.8% in 2017 to 13.0% in 2019, while the prevalence of chronic hypertension increased from 2.0% to 2.3% [10].

Excessive sodium consumption (>5 grams) per day has been proven to lead to a significant increase in blood pressure and is associated with the development of hypertension and cardiovascular complications. Electrolytes such as Sodium (Na^+) and Potassium (K^+) have a crucial role in pre-eclampsia and eclampsia, as they significantly contribute to the function of vascular smooth muscle. Serum Na^+ levels were found to increase significantly in pre-eclampsia patients compared to normal pregnant women [11, 12]. The maladaptation theory explains the disruption in trophoblast interaction with the maternal immune system, which can result in the failure of spiral artery remodeling [13]. Nutritional patterns wherein serum blood Na levels are categorized as high, blood pressure tends to increase [14].

Excessive salt intake regulates the expression of cytokines VEGF, IL-1β, IL-6, and TNF-α and vascular endothelial growth, as evidenced by research results using 40 eight-week-old Sprague-Dawley rats randomly selected and divided into 2 groups. Group 1 received normal food and tap water (normal salt diet) for 30 days and 60 days, while Group 2 was given a high-sodium diet containing 8% NaCl and 1% salt (high-salt diet) for 30 days and 60 days [15].

Hypertension increases the expression of adhesion molecules in endothelial cells. These molecules function as binding tissues between leukocytes and endothelial cells. Adhesion molecules, including e-selectin, p-selectin, sVCAM-1, and sICAM-1, increase in essential hypertension and are independently associated with atherosclerosis. Hypertensive patients have higher levels of e-selectin compared to normotensive participants [16]. MMP-9 plays a role in the degradation of extracellular matrix components. Matrix Metalloproteinase-9 (MMP-9) plays a crucial role in trophoblast invasion into the uterus to create an optimal environment for the embryo [17]. During trophoblast invasion into the decidua, trophoblasts are assisted by proteolytic enzymes such as MMPs. In this case, MMP-2 and MMP-9 are involved in the process of decidua tissue remodeling during pregnancy. Moreover, MMPs also act as membrane receptors and signal transducers. The increase in MMP-9 levels is also influenced by estrogen and progesterone hormones. Matrix Metalloproteinase-9 (MMP-9) is found to increase in normal pregnancy and plays a role in vascular remodeling, angiogenesis, and changes in systemic blood vessels. Additionally, in experiments with mice, MMP-9 also causes relaxation of the aorta and inferior vena cava [5].

METHOD

The research type is True Experimental, with a Post Test only with control group design. This study used pregnant female mice of Mus Musculus strain Balb/C at gestational age of 1 day, which were mated through estrus synchronization technique and had obtained approval from the Ethics Committee of the Faculty of Medicine, Airlangga University (199/EC/KEPK/FKUA/2022). Mus Musculus mice were chosen for this research considering their frequent use in biomedical studies and their genetic similarity to humans, as well as their ability to adapt to laboratory environments.

The inclusion criteria for the mice subjects are: 1) Pregnant Balb/C strain mice (Mus Musculus) at gestational age day 1; 2) Healthy condition indicated by active movement and intact fur, with an average weight of 25-30 grams; and 3) No prior exposure to any chemical substances. The exclusion criteria are: death before the completion of the research treatment or any defects. Dropouts occur if the health condition of the mice deteriorates, or if they die during the study.

The total sample for this study is 72 Balb/c strain Musculus mice, with the following breakdown: 12 mice in the negative control group, 12 mice in the positive control group, 12 mice in treatment group 1, 12 mice in treatment group 2, 12 mice in control group 3, and 12 mice in control group 4. The sampling technique involves breeding female Musculus Balb/C mice by injecting 5 IU Pregnant More Serum Gonadotropin (PMSG), 48 hours later injecting 5 IU Human Chorionic Gonadotropin (HCG). The diagnosis of pregnancy was obtained 17 hours after mating and the presence of a copulatory plug (the plug that covers the vagina of the mouse from the cervix to the vulva) was evaluated to obtain the negative control group, while for the positive control group, pregnant Musculus Balb/C mice are conditioned as a pre-eclampsia model by injecting anti QA-2 from days 1-4 of pregnancy. In this study, each treatment group will be administered salted fish extract containing NaCl at respective doses: group 1: 17.5 mg/day, group 2: dose of 52.6 mg/day, group 3: 87.8 mg/day, and group 4: administered NaCl dose of 52.6 mg/day for 13 days, followed by termination on gestational day 14 by administering ketamine injection.

Extract Material

5 grams of ground salted fish sample was placed into a stoppered Erlenmeyer flask, then added with a mixture of acetone: dichloromethane (50:50, v/v), and left to stand overnight for static extraction process. The extraction result was filtered through a funnel filled with cotton or cleaned glass wool soaked in a mixture of petroleum ether and acetone (4:1, v/v) for eight hours. Subsequently, 25 mL of the organic phase was pipetted into a round-bottom flask, concentrated in a Rotary Evaporator at a water bath temperature of 40°C until nearly dry, and then dried using nitrogen gas. The residue was dissolved in 5 mL of isooctane: toluene (90:10, v/v).

A total of 20 mL of extract was evaporated to near dryness using a Rotary Evaporator at a water bath temperature of 40°C. The resulting residue was dissolved in 20 mL of n-hexane to contain 1 g of the analytical sample. Glass wool, 5 mL of n-hexane, and 1 g of activated silica gel were then added. The mixture was stirred with a stirring rod until homogeneous. The inner walls of the column were rinsed with 2 mL of n-hexane, and the liquid was allowed to flow until the meniscus was just above the silica gel. A total of 2 mL of concentrated extract (equivalent to 1 g of the analytical sample) was introduced into the column, flushed with 3 x 1 mL of n-hexane, and the liquid was allowed to flow until the meniscus was just above the silica gel. Elution was performed with 20 mL of eluent A (a mixture of Ethyl Acetate and n-Hexane, 0.2:99.8 v/v). The first 10 mL of eluate (containing the internal standard) was collected, and the remaining eluate was discarded. Pyrethroids were eluted with 35 mL of eluent B (a mixture of Ethyl Acetate and n-hexane, 10:90 v/v), and the eluate was collected in a round-bottom flask, then 10 mL of the first eluate containing the internal standard was added. It was carefully evaporated to dryness. The residue was dissolved in n-decane to a volume of 1 mL.

Determining the NaCI content

The finely ground sample is accurately weighed to 2 grams and dissolved in hot water until the salt is completely dissolved. The salt extract is then diluted to 100 ml, 10 ml portion of the diluted extract is taken and titrated with 0.1 N $AgNO_3$ using a 5% solution of K_2CrO_4 as an indicator. By using the formula: $\text{NaCl content} = \frac{(\mathbf{Vtx\ N\ AgNO_3} \times \mathbf{Mr\ NaCl} \times \mathbf{100\%}}{\text{Bs}}$.

Immunohitochemical test

For immunohistochemical staining, paraffin is removed by sequentially immersing tissue sections in xylene and ethanol at graded concentrations. The sections are then placed in 3% hydrogen peroxide for 30 minutes and washed for 2 minutes, followed by treatment with 0.025% trypsin for 6 minutes at 37°C. The sections are incubated with primary antibodies (TNF-α, E-selectin, MMP-9) for 30 minutes and washed with PBS three times. The sections are then incubated with secondary antibodies by incubating the sections for 30 minutes, followed by washing with PBS three times for 2 minutes each and incubation with streptavidin/avidin HRP (horse radiash peroxidase) for 30 minutes.

The sections are washed with PBS three times for 2 minutes each, rinsed with distilled water, immersed in Mayer's hematoxylin for 6 minutes, washed with running water, followed by dehydration, clearing, mounting, and observation under a light microscope.

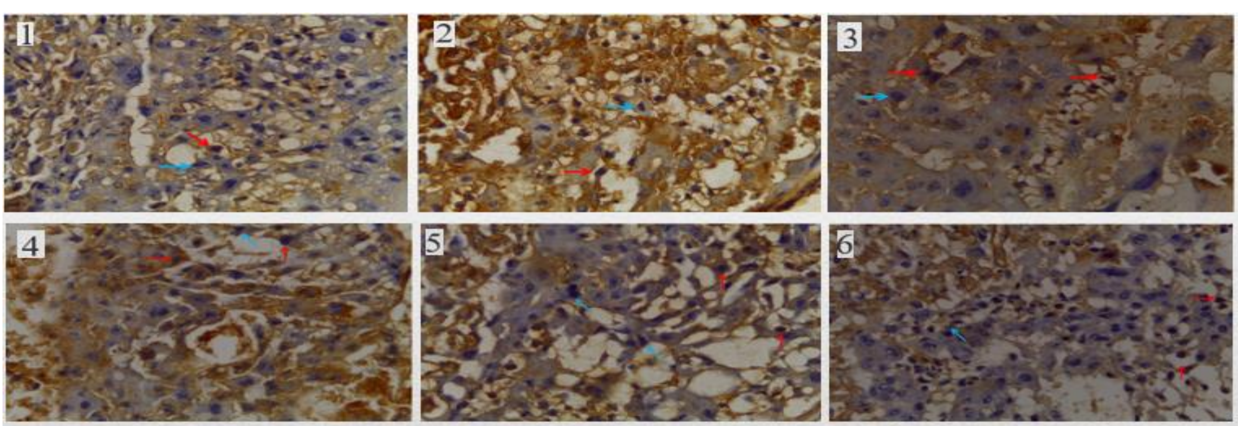
The research variables include: 1) independent variable: Administration of salted fish extract, and dependent variables: TNF-α, e-selectin, and MMP-9. The research instrument is a mouse cage consisting of a sand tray with a wire cover, filled with husks, and equipped with plastic food and water containers measuring 20cm x 30cm x 40 cm, with room humidity at temperatures between 27-28˚C. The mice are fed standard pellet-shaped feed with crude protein, crude fat, calcium, and phosphorus composition, and water is provided daily in special bottles with a daily requirement of 60 ml per mouse. Data analysis for 3 groups or more, the One-way ANOVA or Kruskal-Wallis tests are used. Normality is assessed using the Shapiro-Wilk test with SPSS 22.

RESULT AND DISCUSSION

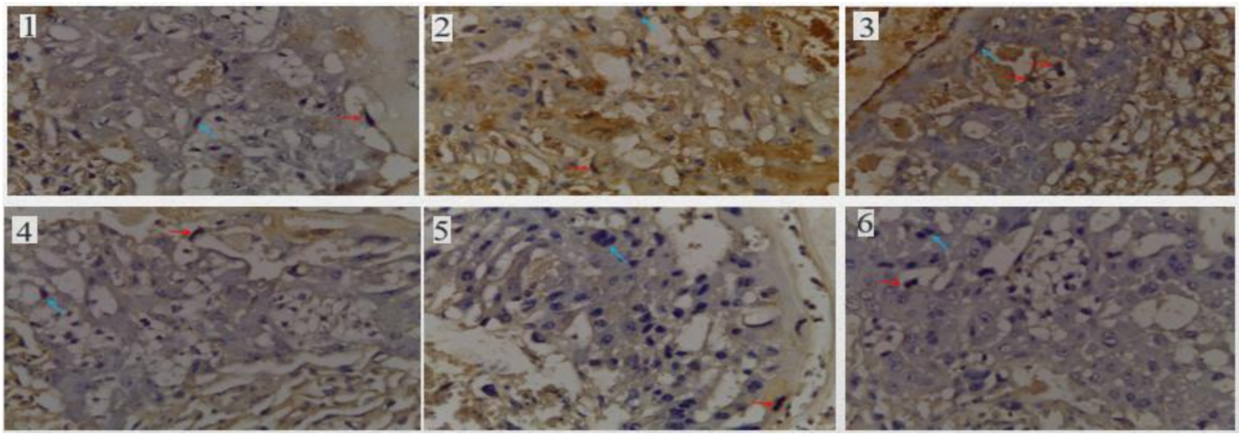
Table 1. The effect of Administration of salted fish extract and NaCI at Various Doses on the Expression of TNF-α, E-selectine, dan MMP-9

Sitokin	Group						p-value
	1 (NC)	2 (PC)	3 (Salted Fish Extract NaCl 17,5)	4 (Salted Fish Extract NaCl 52,6)	5 (Salted Fish Extract NaCl 87,8)	6 (NaCl 52,6)	
TNF-α	1,64^{a}±0,63	6,48^{b} ±1,08	5,38^{b} ±0,82	6,00^{b} ±0,86	6,43^{b} ±0,98	6,44^{b} ±0,98	0,000*
MMP-9	1,63±0,61 1,75^{a}	3,75±1,28 3,50^{c}	2,15±0,78 2,50^{ab}	2,91±0,71 2,85^{bc}	3,66±1,09 4,00^{c}	3,53±1,35 3,20^{c}	0,000*
E-selectin	0,07±0,13 0,00^{a}	1,41±1,27 0,60^{bcc}	1,26±0,52 1,40^{c}	0,50±0,19 0,45^{b}	0,74±0,34 0,60^{b}	0,94±0,55 0,80^{bc}	0,000*

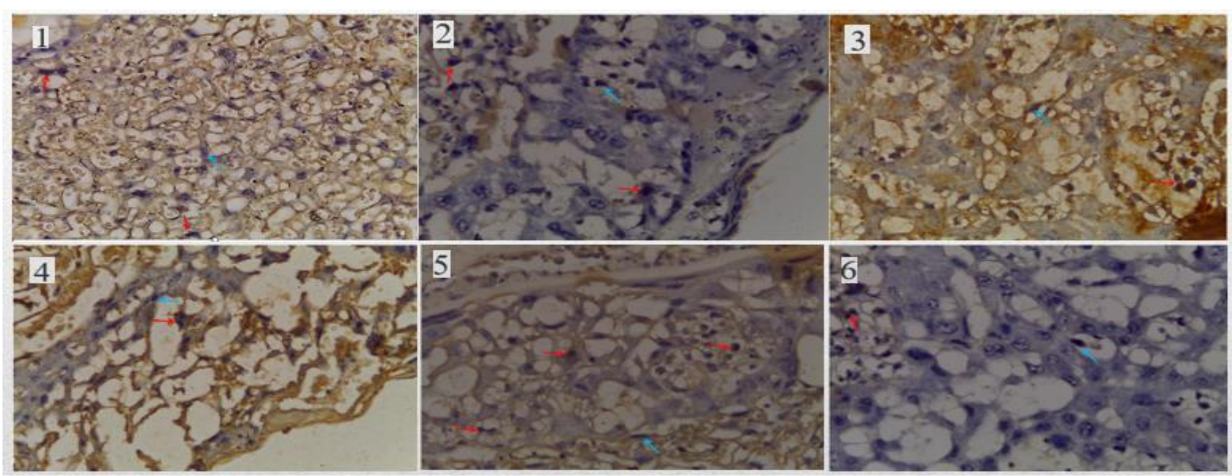
The data represents the mean ± SD. # P<0,05 ^{a,b,c,d,e} shared superscripts on the same row indicate no differences between groups



Picture 1. Light microscope image of TNF-α overexpressed cells stained brown, indicated by red arrows; 1. Negative control, 2. Positive control, 3. Salted fish extract NaCl 17.5 mg, 4. Salted fish extract NaCl 25.6 mg, 5. Salted fish extract NaCl 87.8 mg, 6. NaCl 52.6 mg. Magnification 400x.



Picture 2. Light microscope image of E-selectin overexpressed cells stained brown, indicated by red arrows; 1. Negative control, 2. Positive control, 3. Salted fish extract NaCl 17.5 mg, 4. Salted fish extract NaCl 25.6 mg, 5. Salted fish extract NaCl 87.8 mg, 6. NaCl 52.6 mg. Magnification 400x.



Picture 3. Light microscope image of MMP-9 overexpressed cells stained brown, indicated by red arrows; 1. Negative control, 2. Positive control, 3. Salted fish extract NaCl 17.5 mg, 4. Salted fish extract NaCl 25.6 mg, 5. Salted fish extract NaCl 87.8 mg, 6. NaCl 52.6 mg. Magnification 400x.

Table 1 shows that the mean ± SD score of TNF-α expression was highest in the positive control (PC) group (treated with Anti QA-2) 6.48^{b} ± 1.08, followed by group 6 given NaCl 52.6 mg 6.44^{b} ± 0.98, group 5 given salted fish extract with NaCl content of 87.8 mg was 6.43^{b} ± 0.98, group 4 given salted fish extract with NaCl content of 52.6 mg was 6.00^{b} ± 0.86, group 3 given salted fish extract with NaCl content of 17.5 mg was 5.38^{b} ± 0.82, and the negative control (NC) group was 1.64^{a} ± 0.63. The NC group showed significant differences with all groups, while the PC group, group 3, group 4, group 5, and group 6 showed significant differences only with the NC group, while with other groups, there were no significant differences. ($p=0.000$, $p<0.05$).

The mean ± SD score of MMP-9 expression was highest in the positive control (PC) group (treated with Anti QA-2) 3.75 ± 1.28, followed by group 5 given salted fish extract with NaCl content of 87.8 mg which was 3.66 ± 1.09, group 6 given NaCl 52.6 mg was 3.53 ± 1.35, group 4 given salted fish extract with NaCl content of 52.6 mg was 2.91 ± 0.71, group 3 given salted fish extract with NaCl content of 17.5 mg was 2.15 ± 0.78, and the negative control (NC) group was 1.63 ± 0.61. The NC group showed non-significant differences with group 3 and significant differences with the PC group, group 4, group 5, and group 6. Group 3 showed non-significant differences with group NC and group 4 and significant differences with the PC group, group 5, and group 6. Group 4 showed non-significant differences with group 3 while with other groups, significant differences were observed. Group 5 showed non-significant differences with the PC group and group 6, whereas with group NC, group 3, and group 4, significant differences were observed. Group 6 showed non-significant differences with the PC group and group 5, while with group NC, group 3, and group 4, significant differences were observed. ($p=0.000$, $p<0.05$).

The mean ± SD score of e-selectin expression was highest in the positive control (PC) group (treated with Anti QA-2) 1.41 ± 1.27, followed by group 3 given salted fish extract with NaCl content of 17.5 mg which was 1.26 ± 0.52, group 4 given NaCl 52.6 mg was 0.94 ± 0.55, group 5 given salted fish extract with NaCl content of 87.8 mg was 0.74 ± 0.34, group 4 given salted fish extract with NaCl content of 52.6 mg was 0.50 ± 0.19, and the negative control (NC) group was 0.07 ± 0.13. The NC group showed significant differences with all other groups. The PC group showed significant differences with the NC group and non-significant differences with other groups. Group 3 showed significant differences with the PC group and significant differences with other groups. Group 4 showed significant differences with the NC group and group 3, but non-significant differences with the PC group, group 5, and group 6. Group 5 showed significant differences with the NC group and group 3, and non-significant differences with the PC group, group 4, and group 6. Group 6 showed significant differences only with the NC group and non-significant differences with all other groups. ($p=0.000$, $p<0.05$).

***TNF-α* Expression**

Table 1 shows there is an increase in TNF-α expression in the positive control group with the administration of Anti-QA2 given on days 1-4 of mouse gestation, compared to the negative control group. Anti-QA2 is a specific antibody model capable of binding to the QA-2 protein in mice. This study indicates that the administration of Anti-QA2 at a dose of 40 ng on days 1-4 of mouse gestation results in a decrease in QA-2 expression in trophoblast cells. The decrease in QA-2 expression is followed by an increase in the expression of heat shock protein (Hsp)-70 and vascular cell adhesion molecule (VCAM)-1. The increase in the expression of these molecules is indicative of increased trophoblast apoptosis and endothelial dysfunction. Anti-QA2 can be used for pre-eclampsia models, as demonstrated by the decrease in Placental Growth Factor

(PlGF), Vascular Endothelial Growth Factor (VEGF), and the increase in the anti-angiogenic factor sFlt-1, glomerular endotheliosis, and fetal growth restriction in conception outcomes.

A study in China administered low-dose salt induction food of 5 grams/day or 85.5 mmol Na+/day (WHO standard) and high-salt levels of 15 grams/day (equivalent to 87.8 mg dose in mice) given for 13 days. On day 4, there was an increase in NF-κß levels, and on day 10, there was an increase in IL-8 levels. Proinflammatory cytokines such as TNF-α, IFN-γ, IL-6, and IL-8 can contribute to inflammatory responses through NF-kB activation, a transcription regulator that plays a crucial role in inducing inflammation. Inflammatory conditions caused by proinflammatory cytokine production are a consequence of hypoxia, partly due to hypernatremia-induced changes in blood vessel lumen diameter leading to vasoconstriction, resulting in excessive expression of Hypoxia Inducible Factor-1α (HIF-1α). Levels of proinflammatory cytokines such as IL-6, IL-8, TNF-α, and IFN-γ increase over time in both maternal and fetal vein samples under normoxia or hypoxia. In patients with pre-eclampsia, ischemic placenta can contribute to maternal endothelial cell dysfunction by increasing the synthesis of IL-6, TNF-α, and IL-8.

From the analysis of TNF-α expression, a p-value of 0.000 was obtained, indicating a significant difference between the control group and the treatment group.

MMP-9 Expression

The highest expression of MMP-9 is observed in the positive control (PC) group, followed successively by the treatment groups (3, 4, 5, 6) and the negative control (NC) group, as indicated by the mean±SD values. MMP-9 is one of the crucial members of the matrix metalloproteinase family. MMP-9 can degrade the main component of type IV collagen in the extracellular matrix and is directly involved in the process of trophoblastic epithelial cell attacking the endometrium and embryo implantation. [15]. MMP-9 enzyme has both vasodilatory and vasoconstrictor effects. MMP-9 enzyme can degrade extracellular matrix, causing vasodilatation in the early phase, but does not cause vessel stiffness. Excessive MMP-9 enzyme activity can eventually lead to continuous vessel stiffness, changes in elastic tissue of blood vessels, thus leading to hypertension [5, 17, 24].

Previous clinical research findings have indicated that MMP-9 levels in pregnant women with early preeclampsia are significantly elevated compared to normal pregnancies. This suggests that increased MMP-9 levels become one of the important factors in the pathophysiology of early preeclampsia. Results from studies by Poon et al. (2009) and Lockwood et al. (2014) observed increased MMP-9 levels in pregnancies with preeclampsia complications similar to this study, indicating higher MMP-9 expression in decidual cells, which play a crucial role in preeclampsia [25, 26]. However, a study by Meng (2014) in China showed different results, indicating weak expression of MMP-9 in chorionic trophoblast cells of placenta in early preeclampsia compared to expression in normal pregnancies. Additionally, previous clinical trials have shown that compared to normal women, patients with early preeclampsia have significantly increased serum MMP-9, suggesting that increased serum

MMP-9 levels may be one of the important factors in PE [27].

Some studies indicate increased levels of MMP-9 in preeclampsia, while others describe decreased levels. Collagen, one of the main constituents of MMP-9, can also be implicated in preeclampsia. Increased vascular collagen content leads to rigidity in blood vessels, thereby reducing vascular elasticity. This is associated with increased peripheral resistance in the progression of hypertension [5].

Disruption in blood vessels and molecules related to cell damage (DAMPs) and pathogen damage (PAMPs) can activate an inflammatory response mediated by TLR activation. TLR4 plays a role in inflammatory conditions. NFκß activation, pro-inflammatory cytokine induction, macrophage activation, and dendritic cell activation are roles of TLR-4. In other words, the mechanism of inflammation in neutrophils and macrophages is mediated by direct interaction with TLR4, which enhances TLR signaling and NFκß, followed by increased production of pro-inflammatory cytokines. TLR-4 mediates inflammation through the MYD88 pathway. Pro-inflammatory cytokines resulting from NFκß activation include TNF-α, IL-6, IL-8, and IL-1. TNF-α produces adhesion molecules leading to increased e-selectin and VCAM, causing changes in leukocytes in the blood, thereby increasing MMP-9 [28]. Increased production of IL-6 and TNF-α will activate AT1-AA. Increased AT1-AA will activate ET-1, Angiotensin II, sFlt-1 [29]. Increased AngII by AT1-AA activation will increase MMP-9 in vascular smooth muscle cells [30].

E-Selectin Expression

The highest expression of e-selectin was found in the mean ± SD of the treatment group given salted fish extract containing 17.5 mg of NaCl compared to the control group, which consisted of mice with normal pregnancy without being given salted fish extract or NaCl induction. High salt levels successfully modulated NET production and IL-8 release by neutrophils. These findings are consistent with previous research indicating that high salt levels can induce proinflammatory cytokines through macrophage activation in PE [15].

As a result of exposure to DAMPs, macrophages also produce proinflammatory cytokines such as Interleukin-8 (IL-1), TNF-α, Interleukin-6 (IL-6), and Interleukin-8 (IL-8). Proinflammatory cytokines such as TNF-α, IFN-γ, IL-6, and IL-8 can contribute to the inflammatory response through NF-kB activation, a transcription regulator that plays a crucial role in inducing inflammation [20]. TNF-α is a potent immune response modulator that induces adhesion molecules and neutrophil activation. Interleukin-8 (IL-8), also known as neutrophil chemotactic factor (NCF) and monocyte-derived neutrophil hemotactic factor (MDNCF), is an inflammatory chemokine produced locally in response to tissue damage, crucial for recruiting and activating neutrophils. Interleukin-8 (IL-8) primarily recruits monocytes and neutrophils, thus quickly causing monocytes to migrate and strongly adhere to the single layer expressing e-selectin, allowing them to bind [31]. Adhesion molecules including e-selectin, p-selectin, sVCAM-1, and sICAM-1 increase in essential hypertension and are independently associated with atherosclerosis. Hypertensive patients have higher levels of e-selectin than normotensive participants [16].

The PC group or the preeclampsia model mice injected with Anti-QA2 on days 1-4 of pregnancy had the highest mean ± SD compared to all other groups. The anti-QA2 protein in mice homologous with Human Leukocyte Antigen (HLA)-G in humans. If the expression in trophoblasts is inhibited or low, then the trophoblast cells will be considered non-self antigens that induce antibody formation and desidua. Antibodies that bind to antigens (trophoblasts) will activate the complement pathway, leading to trophoblast lysis and consequent endothelial dysfunction by activating macrophages [32]. Plasma e-selectin concentration can be a marker of endothelial dysfunction or activation [33].

The increase in placental e-selectin expression is associated with increased maternal e-selectin and indicates that placental dysfunction contributes to endothelial damage in preeclampsia. Increased mRNA expression of E-selectin in HUVECs and HEECs endothelial cells after incubation with maternal perfusion found in the placenta of women with preeclampsia [34]. Pro-inflammatory cytokines produced from NFκß activation include TNF-α, IL-6, IL-8, and IL-1. TNF-α induces adhesion molecules leading to increased e-selectin and VCAM, causing changes in leukocytes in the blood, thus increasing MMP-9 [28].

CONCLUSION

There are significant differences in the expression changes of TNF-α, e-selectin, and MMP-9 after the administration of salted fish extract and NaCl in Balb-c strain mice as a model of pregnancy hypertension.

Acknowledgments

The authors would like to thank all those who provided useful suggestions and support in this study.

Funding

This study did not receive a specific grant from a funding agency in the public, commercial, or nonprofit sector.

BIBLIOGRAPHY

1. Alatas H. Hipertensi pada Kehamilan. Herb-Medicine Journal: Terbitan Berkala Ilmiah Herbal, Kedokteran dan Kesehatan. 2019;2(2):27-51; DOI:10.30595/hmj.v2i2.4169
2. Nur AF, Arifuddin A. Faktor Risiko Kejadian Preeklampsia Pada Ibu Hamil Di Rsu Anutapura Kota Palu. Healthy Tadulako Journal (Jurnal Kesehatan Tadulako). 2017;3(2):69-75;https://doi.org/10.22487/htj.v3i2.55.
3. Cerdeira AS, Karumanchi SA. Angiogenic factors in preeclampsia and related disorders. Cold Spring Harbor perspectives in medicine. 2012;2(11); doi: 10.1101/cshperspect.a006585
4. Brennan LJ, Morton JS, Davidge ST. Vascular dysfunction in preeclampsia. Microcirculation. 2014;21(1):4-14; DOI: 10.1111/micc.12079
5. Chen J, Khalil RA. Matrix metalloproteinases in normal pregnancy and preeclampsia. Progress in molecular biology and translational science. 2017;148:87-165; DOI: 10.1016/bs.pmbts.2017.04.001
6. World Health Organization. WHO recommendations on antiplatelet agents for

the prevention of pre-eclampsia. Geneva: World Health Organization; 2021.

7. Karrar SA, Hong PL. Preeclampsia. Treasure Island (FL): StatPearls Publishing; 2023.
8. Bokuda K, Ichihara A. Preeclampsia up to date—What's going on? Hypertension Research. 2023;46(8):1900-7;https://doi.org/10.1038/s41440-023-01323-w.
9. Yayasan Kesehatan Perempuan. Profil Indonesia: Akses universal pelayanan kesehatan seksual dan reproduksi akses Jakarta: Yayasan Kesehatan Perempuan; 2015.
10. Ford ND, Cox S, Ko JY, Ouyang L, Romero L, Colarusso T, et al. Hypertensive disorders in pregnancy and mortality at delivery hospitalization—United States, 2017–2019. MMWR Morbidity and mortality weekly report. 2022;71(17):585–91; DOI: 10.15585/mmwr.mm7117a1
11. Grillo A, Salvi L, Coruzzi P, Salvi P, Parati G. Sodium intake and hypertension. Nutrients. 2019;11(9):1970; DOI: 10.3390/nu11091970
12. Syaputra G, Djanas D. Perbedaan rerata rasio kadar Natrium Kalium Maternal antara preeklamsia berat dan eklamsia. Journal Obgin Emas. 2020;3:31-6; DOI:10.25077/aoj.3.3.31-36.2019.
13. Moffett A, Hiby SE, Sharkey AM. The role of the maternal immune system in the regulation of human birthweight. Philosophical Transactions of the Royal Society B: Biological Sciences. 2015;370(1663):20140071; DOI: 10.1098/rstb.2014.0071
14. Croy BA, Yamada AT, DeMayo FJ, Adamson SL. The guide to investigation of mouse pregnancy: Academic Press; 2013.
15. Anggraini P, Rusdi, Ilyas EI. Kadar Na+, K+, Cl-, Dan Kalsium Total Serum Darah Serta Hubungannya Dengan Tekanan Darah Pada Penderita Hipertensi. Bioma. 2015;11(1):50-66 DOI : 10.21009/Biom
16. Pinheiro MB, Gomes KB, Ronda CR, Guimarães GG, Freitas LG, Teixeira-Carvalho A, et al. Severe preeclampsia: association of genes polymorphisms and maternal cytokines production in Brazilian population. Cytokine. 2015;71(2):232-7; DOI: 10.1016/j.cyto.2014.10.021
17. Espino Y Sosa S, Flores-Pliego A, Espejel-Nuñez A, Medina-Bastidas D, Vadillo-Ortega F, Zaga-Clavellina V, et al. New insights into the role of matrix metalloproteinases in preeclampsia. International journal of molecular sciences. 2017;18(7):1448; DOI: 10.3390/ijms18071448
18. Hikmah EMt, Liben P. Anti-Qa2 animal models for preeclampsia preclinical studies: a pathological elevation of blood pressure and proteinuria. Folia Biologica (Kraków). 2019;67(2):69-78;https://doi.org/10.3409/fb_67-2.07.
19. Luo T, Ji W-j, Yuan F, Guo Z-z, Li Y-x, Dong Y, et al. Th17/Treg imbalance induced by dietary salt variation indicates inflammation of target organs in humans. Scientific reports. 2016;6(1):26767;DOI: 10.1038/srep26767
20. Rahardjo B, Widjajanto E, Sujuti H, Keman K. Curcumin decreased level of proinflammatory cytokines in monocyte cultures exposed to preeclamptic plasma

by affecting the transcription factors NF-κB and PPAR-γ. Biomarkers and Genomic Medicine. 2014;6(3):105-15;https://doi.org/10.1016/j.bgm.2014.06.002.

21. Sjakoer NAA, Permatasari N. Mekanisme Deoxycorticosterone Acetare (DOCA)-garam terhadap Peningkatan Tekanan Darah pada Hewan Coba. El-hayah; 2011.
22. Sinuraya RK, Nisa H, Lokajaya T, Puri TN. Biomarker PlGF/sFlt-1 sebagai Pendeteksi Dini Preeklampsia. Jurnal Farmasi Klinik Indonesia. 2017;6(2):123-34;https://doi.org/10.15416/ijcp.2017.6.2.123.
23. Rodrigo R, González J, Paoletto F. The role of oxidative stress in the pathophysiology of hypertension. Hypertension Research. 2011;34(4):431-40; DOI: 10.1038/hr.2010.264
24. Laskowska M. Altered maternal serum matrix metalloproteinases MMP-2, MMP-3, MMP-9, and MMP-13 in severe early- and late-onset preeclampsia. BioMed research international. 2017;2017; doi: 10.1155/2017/6432426
25. Poon LC, Nekrasova E, Anastassopoulos P, Livanos P, Nicolaides KH. First-trimester maternal serum matrix metalloproteinase-9 (MMP-9) and adverse pregnancy outcome. Prenatal Diagnosis: Published in Affiliation With the International Society for Prenatal Diagnosis. 2009;29(6):553-9; DOI: 10.1002/pd.2234
26. Lockwood CJ, Basar M, Kayisli UA, Guzeloglu-Kayisli O, Murk W, Wang J, et al. Interferon-γ protects first-trimester decidual cells against aberrant matrix metalloproteinases 1, 3, and 9 expression in preeclampsia. The American journal of pathology. 2014;184(9):2549-59;https://doi.org/10.1155/2017/6432426.
7. Meng X-Y, Zhang Q, Li Q, Lin S, Li J. Immunohistochemical levels of cyclo-oxygenase-2, matrix metalloproteinase-9 and vascular endothelial growth factor in papillary thyroid carcinoma and their clinicopathological correlations. Journal of international medical research. 2014;42(3):619-27; DOI: 10.1177/0300060513505485
28. Tolle LB, Standiford TJ. Danger-associated molecular patterns (DAMPs) in acute lung injury. The Journal of pathology. 2013;229(2):145-56;https://doi.org/10.1002/path.4124.
29. LaMarca B, Parrish MR, Wallace K. Agonistic autoantibodies to the angiotensin II type I receptor cause pathophysiologic characteristics of preeclampsia. Gender medicine. 2012;9(3):139-46;https://doi.org/10.1016/j.genm.2012.03.001.
30. Flamant M, Placier S, Dubroca C, Esposito B, Lopes I, Chatziantoniou C, et al. Role of matrix metalloproteinases in early hypertensive vascular remodeling. Hypertension. 2007;50(1):212-8; DOI: 10.1161/HYPERTENSIONAHA.107.089631
31. Apostolakis S, Vogiatzi K, Amanatidou V, Spandidos DA. Interleukin 8 and cardiovascular disease. Cardiovascular research. 2009;84(3):353-60;https://doi.org/10.1093/cvr/cvp241.
32. Laresgoiti-Servitje E. A leading role for the immune system in the pathophysiology of preeclampsia. Journal of leukocyte

biology. 2013;94(2):247-57; DOI: 10.1189/jlb.1112603

33. Zhou CC, Irani RA, Zhang Y, Blackwell SC, Mi T, Wen J, et al. Angiotensin receptor agonistic autoantibody–mediated tumor necrosis factor-α induction contributes to increased soluble endoglin production in preeclampsia. Circulation. 2010;121(3):436-44; DOI: 10.1161/CIRCULATIONAHA.109.902890
34. Mehrabian F, Jazi SMH, Javanmard SH, Kaviani M, Homayouni V. Circulating endothelial cells (CECs) and E-selectin: predictors of preeclampsia. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2012;17(1):15