ORIGINAL ARTICLE

Bali Medical Journal (*Bali Med J*) 2017, Volume 6, Number 3: 543-547
P-ISSN.2089-1180, E-ISSN.2302-2914

Effect of L-Arginine on glomerular endotheliosis improvement in preeclampsia

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Published by DiscoverSys

ABSTRACT

Background: Preeclampsia is the leading cause of morbidity and mortality in pregnant women and the fetus. In preeclampsia, glomerular endotheliosis occurs due to endothelium dysfunction in the kidney. Supplementation with L-Arginine is postulated to improve glomerular endotheliosis. The aim of this study is to investigate the effect of L-Arginine on improving glomerular endotheliosis in an animal model of preeclampsia.

Method: This is an analytical experiment research which was conducted in November 2016 to January 2017 in Biomedical Laboratory Faculty of Veterinary Medicine, University of Airlangga, Surabaya. Thirty pregnant mice (*Mus musculus*) were used in this research. The mice were divided equally into three groups, normal pregnant mice (K1), pregnant mice model of preeclampsia (K2), and preeclampsia pregnant mice treated with L-Arginine (K3). The kidneys were then obtained surgically and examined with immunohistochemistry examination. Data were analyzed by using one-way ANOVA and Post-hoc test.

Results: The mean score of glomerular endotheliosis were 0.44 ± 0.42 for K1, 1.34±0 for K2, and 0.62±0.45 for K3. There was a significant difference between K1 and K2 (p=0.0000), and also between K2 and K3 (p=0,000).

Conclusion: L-Arginine is effective on improving glomerular entoheliosis in a mouse model of preeclampsia.

Keywords: L-Arginine, glomerular endotheliosis, preeclampsia

Cite This Article: Sulistyowati, S., Budiarta, I.N., Soetrisno. 2017. Studying the relationship between spiritual intelligence of nurses and patients' satisfaction with nursing care. *Bali Medical Journal* 6(3): 543-547. DOI:10.15562/bmj.v6i3.672

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Received: 2017-06-09
Accepted: 2017-08-28
Published: 2017-09-1

INTRODUCTION

Preeclampsia is a complication of pregnancy, characterized by high blood pressure and proteinuria that appear after 20 weeks of gestation, and it affects 5 to 10% of all pregnancy. According to the World Health Organization (WHO), 20% of 15 million premature births are related to preeclampsia. In developing countries, the incidence of preeclampsia is higher, and the mortality for both the mother and the premature baby are 20 times higher than in developed country.[1] Although the etiology and pathophysiology of preeclampsia are still unknown, endothelial dysfunction is hypothesized to play a role in the pathophysiology of preeclampsia.[2]

In a normal pregnancy, a well-maintained oxygenation of the placenta is essential. Reactive oxygen species (ROS) originated from high oxygen fluctuation is needed for cell replication, cell proliferation, cell maturation, embryo development, and pregnancy maintenance.[3] The ROS signal is maintained directly by the antioxidants in the body, which also serve as protection. During a normal pregnancy, there is a balance between antioxidants and pro-oxidants. Disturbance of this balance, in which the level of pro-oxidant increased, higher than the body's antioxidant level, is called oxidative stress and this condition will eventually lead to preeclampsia.[5-7]

During pregnancy, the glomerular filtration rate (GFR) can increase between 50 to 70%, in the 13th gestational week it can reach up to 150% of normal, which cause the urea and creatinine level to drop.[8] Renal pathology in preeclampsia is described as endotheliosis or swelling of glomerular endothelial cells. Podocyte, a type of visceral epithelial cells that compose and surrounds the glomerular base membrane, was not considered to be affected by this condition, but a new study revealed that podocytes undergo a structural change and they store an absorbed protein droplet.[9] A glomerulus is an anatomical unit of the kidney that constantly exposes to the ROS. When it reaches a certain concentration, free radicals can impair the function and structure of glomerulus, especially in the mesangial and endothelial cells. The glomerular injury usually is related to excessive activation of ROS which originated from impairment of the natural antioxidant system of an organism, which leads to mediator release, such as cytokine and chemokine that will cause oxidative stress. Oxidative stress will damage the kidney tissue; it especially affects the nephron, and even more, it affects the structure and function of glomeruli and interstitial tubules.[10]

This study used mice (*Mus musculus*) as the study subject, because an invasive technique is

needed to get human kidney samples, and that requires a complex ethical procedure. Thus, the researchers used an animal model, considering that mice are widely used in the biomedical study due to its genetic resemblance with human and its adaptability to survive in the laboratory environment.[11]

L-arginine is one of the essential amino acids, active in the L-form. It is synthesized by the endothelial cell and excreted through the urine. In human, L-arginine supplementation can increase the uteroplacental circulation, lower maternal blood pressure, lower the oxidative stress level, and might have a role in pathophysiology of endothelial dysfunction and preeclampsia.[13]

In normal condition, the human body can synthesize L-Arginine to fulfill the daily requirements, but in stress condition, the need for L-Arginine increases, surpass the production. Upon this condition, L-Arginine in food becomes essential. In the animal model with various kidney impairments, L-Arginine administration has beneficial effects, such as improvement of glomerulosclerosis, improvement of the tubulointerstitial compartment, reduction of macrophage infiltration, improvement of blood flow and GFR, also an improvement in proteinuria.[14] Although preeclampsia is a multifactorial condition, L-Arginine treatment is promising in lowering blood pressure and also helps to maintain the pregnancy, preventing premature birth.[12]

MATERIAL AND METHOD

This was an analytic experimental study which was conducted in November 2016 to January 2017. The impregnation process of mice, creating an animal model for preeclampsia, and administration of L-Arginine treatment to the animal model, and keeping the pregnant mice until the sample collection (16th day of pregnancy) were done in the Experimental Animals Cage, Faculty of Veterinary Medicine, Airlangga University. While the paraffin block preparation and immunohistochemistry examination were done in Biomedical Laboratory, Faculty of Veterinary Medicine, Airlangga University. Study on the animal model was conducted using mouse kidney which met the inclusion criteria. The mice were female mice of the species *M. musculus*, Swiss strain, that were obtained from Veterinaria Farma Surabaya. In this experiment, the female mice used were three months old, healthy, and weighed around 20-25gr. The mice were then dissected, to obtain the kidneys which then processed into paraffin blocks, and then later were stained using Hematoxylin-Eosin (HE) stain.

There were 30 mice used in this study, divided into three groups; normal pregnant mice (K1), pregnant mice model of preeclampsia (K2), and preeclampsia pregnant mice treated with L-Arginine (K3).

The mice impregnation process were done through estrous synchronization, by injecting the mice with 5 IU of Pregnant More Serum Gonadotropin (PMSG) hormone, then 5 IU of Human Chorionic Gonadotropin (hCG) 48 hours later. Female mice were then mated in a mono making way, with a single healthy 7-months-old male mouse, weighted ±60 gram. The female mice that have been undergone the estrous synchronization process were then placed one by one in the same cage with the male mouse to mate. A mouse was diagnosed pregnant if there was a copulatory plug (a plug in the vagina, from the cervix to vulvae).

On the first day of pregnancy, all samples were divided into three groups; 10 normal pregnant mice (K1) were kept without any interventions, 20 mice (K2 and K3. Each group consisted of 10 mice) were administered with 10 ng Qa-2 intravenously during the 1st to 4th day of pregnancy, so that the mice would be preeclampsia model.[11] The group of mice model of preeclampsia (K2) was given 200 mg/kg of body weight of L-arginine during 7th to 15th day of pregnancy. On the 16th day of pregnancy, all mice were terminated. Pregnant mice were euthanized with ketamine and then continued with necropsy. After the abdominal cavity was opened, kidney samples were obtained and then placed in the pot containing 10% neutral buffered formalin. The samples were harvested on the 16th day of pregnancy, assuming that it is equal to the second trimester of human pregnancy, when the manifestations of preeclampsia begin to show in human.

At the beginning of histology preparation, the kidneys were fixated by using 10% neutral buffered formalin solution. Then the kidney samples were cut and collected in a specimen container made of plastic. Then the process continued into dehydration process with gradually increased alcohol concentration, started at 70%, then 80% and 90%. Absolute alcohol Labsolute II was used in this process, and each process took two hours. The specimens were then cleared with xylol, embedded in paraffin, and stored in the refrigerator. By using microtome, the paraffin blocks were cut as thick as 5-6 µm. The slices then floated in warm water (60°C) to stretch the tissue, so that the tissue would not fold. Specimens were then placed in object glass and then stained with Hematoxylin and Eosin (HE) stain. The specimens were examined under the Nikon eclips Ci microscope, at 400x magnification, with optilab viewer 2.2.

Counting of the cells that undergo the glomerular endotheliosis was done in five fields of view from each paraffin block specimen. The parameter

Published by DiscoverSys | *Bali Med J* 2017; 6(3): 543-547 | doi: 10.15562/bmj.v6i3.672

to determine glomerular endotheliosis was based on criteria from Isaac and Swensson, by finding the histopathologic changes such as podocyte vacualization and mesangial cell proliferation. The number of cells that underwent glomerular endotheliosis in each specimen was recorded and then added to determine the score.[15] Data were analyzed by using one-way ANOVA test and post hoc test, with significance level at 0.05 (confident interval 95%).

Ethical clearance

Ethical clearance was obtained from Research Ethics Committee Faculty of Veterinary Medicine, Airlangga University, Number 648-KE, 15 November 2016.

RESULTS

The mean scores of glomerular endotheliosis in each group were presented in Table 1. The mean score of glomerular endotheliosis for each group were: 0.44±0.42 for K1, 1.34±0.53 for K2, and 0.62±0.45 for K3. There was a significant difference between KI and K2, and between K2 and K3 (p = 0.000). While between K1 and K3, there was no significant difference (p = 0.40).

The histopathology appearances of glomerulus that represent each group were presented in Figure 1. In normal pregnant mice, there was no mesangial/matrix proliferation (yellow arrow). There was also no podocyte vacuolization (blue arrow), and the capillary walls appear normal (green arrow).

Table 1 Mean score of glomerular endotheliosis in each mice group

Variable	Group			
	K1 (N=10)	K2 (N=10)	K3 (N=10)	*p*
Mean score of glomerular endotheliosis	0.44±0.42	1.34±0.53		0.00*
		1.34±0.53	0.62±0.45	0.00*
	0.44±0.42		0.62±0.45	0.40

*significant at p<0.05

In mice model of preeclampsia (K2), a massive accumulation of inflammatory cells can be observed in the kidney interstitial (green arrow). There is also necrosis in the tubules, and the border between each tubule become unclear (black arrow). Mesangial/matrix proliferation can also be observed (blue arrow). There is also podocyte vacuolization, marked by abnormal cavity or vesicle filled with fluid in the cytoplasm, which transforms the visceral layer of epithelial cells of Bowman capsule (yellow arrow).

In the preeclampsia model treated with L-Arginine, a few observations can be made. There is a proliferation of mesangial/matrix (blue arrow) that causes narrowing of the blood vessels (black arrow). Podocyte vacuolization can also be observed (yellow arrow). In this specimen, although the mesangial cell proliferation and podocyte vacuolization can be observed, although it is not as massive as observed in the preeclampsia model not treated with L-Arginine. It is visible from the less massive proliferation of mesangial or mesangial matrix (close to normal) and podocyte vacuolization that there is an improvement of glomerular endotheliosis in this specimen.

DISCUSSION

In this study, the mean score of endotheliosis in K1 and K2 group has a significant difference that showed the endotheliosis difference in the kidney of normal pregnancy and preeclampsia due to endothelial dysfunction. The mean of endotheliosis in group K2 and K3 also has a significant difference. This differences showed that L-Arginine treatment in preeclampsia has a positive impact on improving the glomerular endotheliosis, approaching normal condition.

L-Arginine acts as a precursor of nitric oxide (NO), that transformed into NO and L-citrulline by nitric oxide synthase (NOS), which can prevent the preeclampsia. Combined supplementation of L-Arginine and antioxidant is related to the

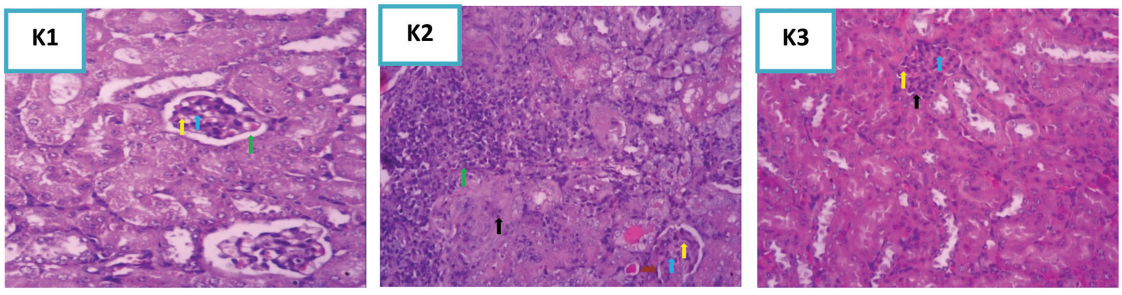

Figure 1 The histopathological comparison between the kidney samples obtained from normal pregnant mice (K1), preeclampsia model (K2), and preeclampsia model treated with L-Arginine (K3)

significantly low incidence of preeclampsia.[16] Considering that L-Arginine is a widely available food supplement, L-Arginine is a potential treatment in preventing preeclampsia.[17] The concentration of L-Arginine had been proved to be significantly low in women with preeclampsia when compared to a normal pregnant woman. A study in an animal model showed that the L-Arginine – NO system undergoes misregulation during pregnancy. Hypertension, proteinuria, intrauterine growth restriction, and glomerular damage can happen due to blockade of NO synthesis, while on the other hand, L-Arginine supplementation can improve hypertension that caused by inhibition of NO synthesis.[12] Thus, L-Arginine could be a new treatment option for hypertension in pregnancy, to prevent pre-eclampsia in high-risk women.

Preeclampsia is caused by maladaptation of spiral artery which caused hypoperfusion of placenta, triggers activation or extensive dysfunction of maternal endothelial cells in blood vessels of some maternal organs. Maternal endothelial damage can be visualized clearly in the kidney, appears as pathological changes, which is typical of preeclampsia. These changes are specific in pregnancy, and not observed in the normal pregnant mouse because preeclampsia triggers the inhibition of NOS, which can be seen from the lower activity of NOS in the kidney of pregnant mice. Preeclampsia is related to kidney endothelial dysfunction that manifests morphologically as glomerular endotheliosis.[18]

Structural changes of the kidney's endothelial cells are similar to the characteristic glomerular lesion found in human preeclampsia. All of these structural changes play a role in kidney dysfunction. Maternal vascularization of those with preeclampsia, oxidative stress, vascular function changes, and increased production of a placental factor, which is known to induce ROS production. Oxidative stress and decreased capacity of local antioxidant activity can be one of the factors that lead to renal dysfunction, proteinuria, and renal pathology in preeclampsia.[19] Antioxidants act as a physiological protective agent that prevents oxidative damage caused by high level of ROS.[5] L-Arginine supplementation eliminates high blood pressure and endothelin response towards chronic elevations of plasma soluble FMS-like tyrosine kinase-1(sFlt-1) level in pregnant mice.[20] The role of decreased NO synthesis in preeclampsia is supported by data which showed that L-Arginine supplementation lowers the blood pressure as a response towards placental ischemia in both pregnant mice and women.[16,20] A study showed that L-Arginine might improve proteinuria, hyperuricemia, and glomerular endotheliosis condition in preeclampsia syndrome. These findings indicate that L-Arginine supplementation in preeclampsia is significant with the present study, that L-Arginine has a positive impact towards the improvement of glomerular endotheliosis.[14]

CONCLUSION

L-Arginine has positive effects on the improvement of glomerular endotheliosis in pregnant mouse model of preeclampsia.

CONFLICT OF INTEREST

The authors state that there is no conflict of interest related to this study, the author, and the publication of this article.

ACKNOWLEDGMENTS

The authors would like to express their gratitude to the Head of Veterinary Reproduction Laboratory, Faculty of Veterinary Medicine, Airlangga University.

REFERENCES

1. Sánchez-Aranguren LC, Prada CE,Riaño-Medina CE, Lopez M. Endothelial dysfunction, and preeclampsia: the role of oxidative stress. Front Physiol. 2014;5:372.
2. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, et al. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. The Lancet. 2012;379(9832):2151–61.
3. Mutinati M, Piccinno M, Roncetti M, Campanile D, Rizzo A, Sciorsci R. Oxidative stress during pregnancy in the sheep. Reprod Domest Anim. 2013 Jun;48(3):353-7.
4. Yang X, Guo L, Li H, Chen X, Tong X. Analysis of the original causes of placental oxidative stress in normal pregnancy and pre-eclampsia: a hypothesis. J Matern Fetal Neonatal Med. 2012;25(7):884-8.
5. Lappas M, Mitton A, Permezel M. In response to oxidative stress, the expression of inflammatory cytokines and antioxidant enzymes are impaired in placenta, but not adipose tissue, of women with gestational diabetes. J Endocrinol. 2010;204(1):75-84.
6. Matsubara K, Matsubara Y, Hyodo S, Katayama T, Ito M. Role of nitric oxide and reactive oxygen species in the pathogenesis of preeclampsia. J Obstet Gynaecol Res. 2010;36(2):239-47.
7. Kalyanaraman B. Teaching the basics of redox biology to medical and graduate students: Oxidants, antioxidants, and disease mechanisms. Elsevier. 2013;1(1):244-257
8. Cheung KL, Lafayette RA. Renal Physiology of Pregnancy. Adv Chronic Kidney Dis. 2013;20(3):209–14.
9. Jim B, Jean-Louis P, Qipo A, Garry D, Mian S, Matos T, et al. Podocyturia as a diagnostic marker for preeclampsia amongst high-risk pregnant patients. J Pregnancy. 2012;2012:984630.
10. Tamay-Cach F, Quintana-Pérez JC, Trujillo-Ferrara JG, Cuevas-Hernández RI, Del Valle-Mondragón L, García-Trejo EM, et al. A review of the impact of oxidative stress and some antioxidant therapies on renal damage. Ren Fail. 2016;38(2):171-5.
11. Sulistyowati S, Abadi A, Hood J, Soetjipto S. The Influence of Low HLA-G Protein Expression on HSP-70 and VCAM-1 Profile in Preeclampsia Indonesian J Obstet Gynecol. 2010;34(4):185-90.

12. Gui S, Jia J, Niu X, Bai Y, Zou H, Deng J, Zhou R. Arginine supplementation for improving maternal and neonatal outcomes in the hypertensive disorder of pregnancy: a systematic review. J Renin Angiotensin Aldosterone Syst. 2014;15(1):88-96.
13. Camacho E, Silva JA, Matos MG, Garrido MR, Israel A. Actividad de las enzimas antioxidantes en el rinon de la rata con preeclampsia experimental. Archivos Ven Farmacol Ter. 2011;30(3):44-50.
14. Adhi JS. Perbaikan Fungsi Ginjal Pada Preeklampsia Setelah PemberianL-Arginine per oral: Kajian Tentang Proteiuria, Hiperurisemia dan endoteliosis Glomerular pada Rattus norvegicus Strain Water. [Thesis] Yogyakarta, Indonesia: Universitas Gajah Mada; 2006.
15. Wide-Swensson D, Strevens H, Willner J. Antepartum percutaneous renal biopsy. Int J Gynaecol Obstet. 2007;98(2):88-92.
16. Vadillo-Ortega F, Perichart-Perera O, Espino S, Avila-Vergara MA, Ibarra I, Ahued R, et al. Effect of supplementation during pregnancy with L-Arginine and antioxidant vitamins in medical food on pre-eclampsia in high risk population: randomised controlled trial. BMJ. 2011;342:d2901.
17. Johal T, Lees CC, Everett TR, Wilkinson IB. The nitric oxide pathway and possible therapeutic options in pre-eclampsia. Br J Clin Pharmacol. 2014;78(2):244-57.
18. Ulrich Förstermann, William C. Sessa. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;33(7):829–837.
19. Elsa C, Matos MG, Garrido MR, Israel A. Rat Kidney Antioxidant Enzyme Activities In Experimental Preeclampsia. International Journal of Current Medical And Pharmaceutical Research. 2015;1(7)104-109.
20. Murphy SR, LaMarca B, Cockrell K, Arany M, Granger JP. L-Arginine supplementation abolishes the blood pressure and endothelin response to chronic increases in plasma sFlt-1 in pregnant rats. Am J Physiol Regul Integr Comp Physiol. 2012;302(2):R259-63.