Effect of Vitamin D Supplementation on Aorta Diameter of Pregnant Wistar Rats (*Rattus Norvegicus)*

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Abstract

Background: Hypertension is one of the clinical signs of preeclampsia or eclampsia, which is estimated to be seven times higher in developing countries than in developed countries. It can be caused by the differences in nutritional status and nutritional intake, one of which is vitamin D. Vitamin D deficiency (VDD) has a negative impact on pregnancy. It increases the risk of preeclampsia, long-term hypertension, miscarriage, and vascular disorder. Vasoconstriction as the beginning of preeclampsia can be related to the diameter of the aorta which is the largest blood vessel in the human body.
Purpose: Determine the effect of vitamin D intake on the aorta diameter of pregnant Wistar rats (*Rattus norvegicus*).
Method: True experimental with post-test only control group design. Research on pregnant Wistar rats (*Rattus norvegicus*) was divided into control and treatment groups. The treatment group of pregnant Wistar rats was given a standard diet during pregnancy and given a dose of 1000 IU/day (human doses) or 18 IU/day (rat doses) of vitamin D on days 1 to 17. On the 18th day, surgery was performed to take the aorta, and its diameter was measured.
Results: The mean aorta diameter of the control group was 1742,04 µm and the treatment group was 1889,30 µm.
Conclusion: There is a clinical inclination that the mean aorta diameter of pregnant Wistar rats (*Rattus norvegicus*) in the treatment group given 18 IU vitamin D is greater than the control group.

Keywords: Vitamin D, aorta diameter, pregnancy

BACKGROUND

Hypertension is one of the clinical signs of preeclampsia and eclampsia. According to WHO, preeclampsia incidence is estimated to be seven times higher in developing countries than in developed countries.(Perkumpulan Obstetri Ginekologi & Indonesia, 2016)The difference in nutritional status or intake can cause it.(Aditiawarman et al., 2020; Retnosari et al., 2015) One of the nutritional status that can affect pregnancy is vitamin D.(Lo et al., 2019)

Vitamin D is a white crystal compound that is soluble in oil and fat.(Simanjuntak & Elly, 2021) Vitamin D has functions of immunomodulation, anti-inflammation, blood pressure regulation, antiangiogenic factors on the placenta, bone metabolism, and acts as a natural antioxidant that can pass the blood-brain barrier.(Ardiaria, 2020; Retnosari et al., 2015; Schulz et al., 2017) Vitamin D deficiency in pregnant women in Asia reaches 80%. In Jakarta, Indonesia, especially, has a high prevalence of vitamin D deficiency, estimated at 90%.(Bardosono, 2016; Octavius et al., 2023) Having vitamin D deficiency during pregnancy can affect pregnancy. The maternal has a risk of preeclampsia, long-term hypertension, stroke, miscarriage, and vascular disorders. Besides, the fetus has a risk of developing several abnormalities like intrauterine fetal death (IUFD), intrauterine growth restriction (IUGR), premature birth, solution placenta, fetal distress, and low birth Weight (Fox et al., 2019; Irianti et al., 2020; Octavius et al., 2023)

Preeclampsia has clinical signs like hypertension and proteinuria which occurs on 20 or more weeks of pregnancy.(Lopez-Jaramillo et al., 2018) Meanwhile, vasoconstriction or vasospasm is the beginning of preeclampsia where there is a condition with contraction reducing the diameter of blood vessels in response to a stimulus.3,14(Retnosari et al., 2015; *VASOSPASM Definition & Meaning - Merriam-Webster*, n.d.)–16 Vasoconstriction can be related to the diameter of the aorta which is the largest blood vessel in the human body. (Saadoun et al., 2021; Shahoud et al., 2023) Vitamin D can reduce the risk of reducing blood vessels by preventing endothelial dysfunction and blood vessel resistance. Furthermore, it can reduce several placenta antiangiogenic factors. Vitamin D can suppress the renin gene code so that renin is not formed. This can make the renin-angiotensin system not active and blood pressure not elevated.(Behjat Sasan et al., 2017; Berraies et al., 2015; Palacios et al., 2024; Retnosari et al., 2015; Schulz et al., 2017) However, some research shows that vitamin D did not affect endothelial cells, blood pressure, and the renin-angiotensin system.(Borgi et al., 2017; Cremer et al., 2018;

Tabrizi et al., 2018)

Based on that explanation, the researcher has investigated the effect of vitamin D 18 IU/day (rat doses) on the aorta diameter of pregnant Wistar rats (Rattus norvegicus) which is equivalent to 1000 IU/day in human doses. The dose of 1000 IU/day was chosen because this dose is still in the safe category and can be recommended without the need to measure serum vitamin D levels because the higher dose is for symptomatic antenatal care (Mithal & Kalra, 2014; Pérez-López et al., 2020)

METHODS

This research uses true experimental and post-test only group design. Research on pregnant Wistar rats (*Rattus norvegicus*) was divided into control and treatment groups. The treatment group of pregnant Wistar rats was given a standard diet during pregnancy and given a dose of 18 IU/day of vitamin D on days 1 to 17. Doses are converted using the Laurence- Bachrach table human dose 70 kg with rats 200 grams and factors conversion or multiplication coefficient of 0.018. Next, the weight dose of vitamin D in rats was 0.001 grams which is determined through comparison between the weight of 1 vitamin D tablet in humans is 0.084 grams, and 1 tablet in rats and a dose of 1000 IU in humans with 18 IU in rats. Every weight of the 0.001 gram dose given to rats will be dissolved in a solution of carboxymethyl cellulose (CMC) as much as 0.05 ml. The final dose weight was given to rats in the amount of 0.002 grams dissolved in 0.2 ml of CMC because Another 0.001 gram in the final dose and another 0.1 ml in the CMC as a dose and reserve solution when given to rats.

On the 18th day, surgery was performed to take the aorta, and its diameter was measured. Based on WHO regulations, the minimum sample for this research is 5 rats in each group. To calculate the possibility of a dropout sample is 10%, it can be concluded that sample each group is:

$$n_{d_0} = \frac{n}{1 - do^2}$$

$$n_{d_0} = \frac{5}{(1 - 0{,}1)^2}$$

$$n_{d_0} = 6{,}2 \approx 7$$

After doing the calculations, a total of 14 pregnant Wistar rats were needed for the research. Each group contained 5 samples of pregnant Wistar rats and 2 spares pregnant Wistar rats. Inclusion rats were adapted for 7 days with feed and drink standard ad libitum. The ratio for the mouse mating process is female: male = 2:1. Thus, 7 male Wistar rats are needed to make 14 female Wistar rats pregnant. The 1st day of pregnancy is indicated

by vaginal plaque. The early pregnant rats were the treatment group and the others that were not pregnant yet were mating with the male rats again. Furthermore, the rats were divided into 2 groups by random sampling and their weight was measured.

The surgical procedure was held in the Animal Laboratorium, Faculty of Medicine, Diponegoro University on the 18th day of pregnancy. The ascendants aorta is picked up and placed in buffer neutral formalin (BNF) 10%.

This study has obtained ethical clearance from the Health Research Ethics Commission of the Faculty of Medicine Diponegoro University No.72/EC-H/KEPK/FK-UNDIP/VII/2023, a research permit from the Faculty of Medicine Diponegoro University No. 225/UN7.F4.5.1/PP/VI/2023.

RESULTS

Normality of aorta diameter sample data was tested with Shapiro-Wilk. Normal distribution data if the p-value is > 0,05. Based on the table below, the control group has p=0,087, and the treatment group has p=0,402. Therefore, both groups have normal distribution data.

Table 1. Shapiro-Wilk, Normality Test of Aorta Diameter (µm)

Group	Shapiro - Wilk		
	Statistic	df	*P*
Control	0,834	7	0,087
Treatment	0,905	6	0,402

**P-value >0,05 as significant*

The results were obtained from the normal distribution in normality test, followed by a different test using the Independent T-Test.

Based on the table below, the mean aorta diameter of the control group is 1742,04 µm. Hence the mean aorta of the treatment group is 1889,30 µm. It represents that the mean aorta diameter of the treatment group is bigger than the control group. Nevertheless, based on this test, it gives p=0,584 (p>0,05) it can be interpreted that the difference of the mean aorta diameter of the control and treatment group is not significant.

Table 2. Aorta Differential Test for Each Group

	Group	n	Mean ± SD	*P*
Pregnant Wistar Rats	Control	7	1742,04 ± 564,78	0,584
	Treatment	6	1889,30 ± 318,22	

**P-value <0,05 as significant*

Figure 1, in code A, the aorta diameter of the control group which was not given vitamin D appears to have converted walls. Code B is the treatment group given 18 IU of vitamin D. The longitudinal blue line shows the aorta diameter (lumen) that was observed and

described in this study. The red line is the total diameter.

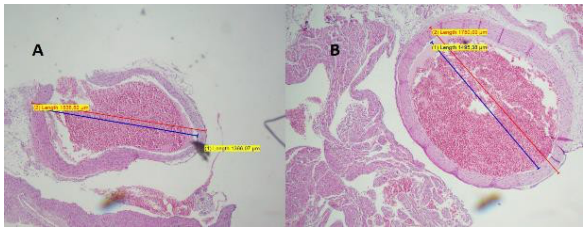

Figure 1. A: aorta diameter of the control group; B: aorta diameter of the treatment group.

DISCUSSION

Aorta diameter as a part of the main blood vessels (aorta) in the human body has mechanisms and structures that are related to several things. It can be the angiogenic or vasculogenic factor that involves endothelial cells in pregnant women and is not related to vitamin D supplementation.(Ali et al., 2021; Crivellato, 2011; National Cancer Institute, 2018) The function of vitamin D on endothelial cells is affected by its doses, duration, or pathological condition of the sample.(Kim et al., 2020)

Vitamin D in this research did not affect and correlate with aorta diameter in pregnant Wistar rats. It is in line with previous research which has been done. Vitamin D did not affect endothelial cells, which is one of the components of the aorta diameter wall.(Tabrizi et al., 2018) Tabrizi, et al (2021) said that there is no effect of vitamin D >4000 IU/day supplementation on endothelial.(Tabrizi et al., 2018) Furthermore, Subramanian, et al (2021) said that the results of their research showed that there was no effect of using vitamin D at a dose of 16,800IU/week to reduce blood pressure in pregnant women.(Subramanian et al., 2021) The incidence of hypertension in pregnant women where there was a reduction of the diameter of the blood vessels also did not show any correlation with vitamin D supplementation in any trimester.(Forde et al., 2021; Touyz et al., 2018)

The sample condition is a factor that can influence research results. Some studies suggest that the renin-angiotensin system may provide feedback on vitamin D(Shroff et al., 2012) The mechanism of that system can be affected by chronic kidney disease which can potentially accelerate the progressivity of that disease.(Vaidya & Williams, 2012) There is a result of research that said the possibility of angiotensin II will reduce alfa- hydroxylase enzyme, so it can reduce the activation of vitamin D in the renin- angiotensin system. The damage of vitamin D receptor in the kidney is in line with the increase of renin and angiotensin that can direct to reducing the diameter of blood vessels, which is related to hypertension.(Shroff et al., 2012)

The temperature of the environment can affect the physiology of rats, like the heartbeat or blood pressure.(Swoap et al., 2004) The optimal temperature for pregnant

rats is 24 – 26^{0}C.(Department of Primary Industries, n.d.) However, the environmental temperature in this research is 22 – 26^{0}C. Temperature that is not suitable can increase stress in rats. It can change the metabolism or immunity.(Gaskill et al., 2013) Furthermore, the stress pregnant rats can also reduce the diameter of blood vessels or higher hypertension than the rats that were not stressed.(Takiuti et al., 2002)

Limitation of this study are determining the first day of pregnancy in Wistar rats. It was challenging because no sperm was visible in several observation samples. Therefore, researchers needed to observe periodically. Furthermore, there are limitations in knowing the pathological conditions in rats that cannot be detected by general inspection. For example, researchers who cannot know the vitamin D status or diseases that rats may suffer from.

CONCLUSION

It was concluded that there is a clinical inclination that the mean aorta diameter of pregnant Wistar rats (*Rattus norvegicus*) in the treatment group given 18 IU vitamin D is greater than the control group. However, there was no difference in supplementation of 18 IU vitamin D on aorta diameter pregnant Wistar rats (*Rattus norvegicus*) in the treatment group with control group. It would be better for further researchers to include more variables, the number of samples, and methods to obtain more accurate and representative research results. For example, researchers might consider using graded doses as variables, using mice as sample, and using a specialized rat test pack method to accurately determine the initial day of pregnancy in Wistar rats.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

AZK; writing manuscript, analyzing data, MBA; conceptualization, FEP; validation, RDC; review.

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