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Urine Protein, Creatinine, and Uacr Level in Pregnant *Mus Musculus* Injected by Anti Qa2 as Endothelial Dysfunction Model to Induce Preeclampsia

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Keyword: Protein, Creatinine, UACR, Urine, Endothelial Dysfunction

Abstract: Preeclampsia is a condition which was identified by diastol blood pressure ≥ 90 mmHg in 20 weeks of pregnant, and blood pressure in < 20 weeks of pregnant was still < 90 mmHg. Beside hypertension, we also find proteinuria in preeclampsia after 20 weeks of pregnant. Preeclampsia could cause low renal function that was indicated by creatinine and UACR level. Preeclampsia happened from endothelial dysfunction. Endothelial dysfunction in Mus musculus could be made by injecting anti QA2. This research was to analyze urine protein, creatinine, and UACR level in endthelial dysfunction model in pregnant Mus musculus. The result was dose of anti QA2 could cause the increasing of urine protein and UACR level, but not to urine creatinine level. The conclusions were there was significant differences of urine and UACR protein level in pregnant Mus musculus that was injected by anti QA an endothel dysfunction model that induced preeclampsia, but not to urine creatinine level. The suggestions were preeclampsia must be detected, prevented and treated as soos as possible, to prevent mechanism of endothel dysfuncyion in preeclampsia that could cause low renal function.

1. INTRODUCTION

According to *preeclampsia community guideline* (PRECOG), preeclampsia is a condition which is identified by diastol blood pressure ≥ 90 mmHg in 20 weeks of pregnant, and blood pressure in < 20 weeks of pregnant was still < 90 mmHg. Beside hypertension, we also find proteinuria in preeclampsia after 20 weeks of pregnant. Proteinuria is protein in urine ≥ 300 mg/litre or ≥ +1 in dipstick test, ratio of protein/creatinin ≥ 30 mg/mmol, or excretion of protein in urine ≥ 300 mg in 24 hours (Milne et. al., 2005).

Signs of preeclampsia are: (Hladunewich dkk., 2007)

a. Hypertension (blood pressure ≥ 140/90 mmHg). Hypertension in preeclampsia happens because of disturbances of vasoactive factors such as vasoconstrictor (endothelin, tromboxan) that are higher than vasodilator (nitric oxide/NO, prostacyclin).
b. Low GFR (glomerulus filtration rate): it happens because the structure of glomerulus changes after vasoconstriction. This condition is showed by urine albumine and creatinine ratio (UACR) level.
c. Proteinuria: it shows the difference between preeclampsia and the other hypertension types. Proteinuria happens because of the disturbances in glomerulus filtration barier.
d. Coagulopathy and HELLP syndrome: endothelial dysfunction in preeclampsia causes light coagulopathy with high trombocyte numbers, slow clothing time, and low antrombine III. HELLP syndrome can happen

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in 10% of severe preeclampsia. It increases plasma concentration and trombocyte activation.

Data of Indonesian Health Ministry in 2010-2013 showed that hypertension was the second cause of maternal mortality in Indonesia after bleeding (Kemenkes RI, 2014). High rate of preeclampsia must be followed by effective preventive and treatment that still need a lot of researches.

Some researches for treatment and prevention of preeclampsia can not be done in human. So we need animal model that will be similar to preeclampsia. The researches about animal model of preeclampsia were very variated and need to be confirmed with cellular and clininal examanations.

One of researches about preeclampsia animal model was endothelialial dysfunction model. It was made by Sulistyowati et. al. (2010) as induction of preeclampsia. That endothelial dysfunction model was done by injecting anti QA2 (anti Human QA Lymphocyte Antigen 2 Region). It blocked QA2 expression in placenta.

Placental QA2 expression was homolog to human leucocyte antigen-*G* expression (HLA-G) in human. Low HLA-G in trophoblast was a predictor to endothelial dysfunction in preeclampsia. That reasearch showed that endotel dysfunction model in Mus musculus cauesd HSP70, VCAM-1 and matrix metalloproteinase (MMP9) profiles that were similar with women with preeclampsia (Sulistyowati et. al., 2010). That research did not examine clinical examanations that appeared from endothelial dysfunction.

The goal of this research was making endothelial dysfunction in pregnant Mus musculus that was injected by anti QA2 and confirming urine examanation (protein, creatinine, and UACR urine) as one of preeclampsia clinical manifestations because of endothelial dysfunction and low renal function.

2. MATERIAL DAN METHOD

This research was true experimental with post test only with control group design. This research used female *Mus musculus* that was mated by male *Mus musculus*. Female Mus musculus with positive vaginal plug were used in the research. The vaginal plug was the sign those female and male *Mus musculus* were mated and the pregnant was called 0 day.

Mus musculus that were used must be 3 months, healthy, bodyweight 15-25 grams, well moving, no wound found in the body, and clear eye. This research used 3 pregant *Mus musculus*/groups. The duration of research was 2 weeks, consisted of acclimatization, mating female and male Mus musculus, intervention, and termination.

All of female *Mus musculus* were injected by pregnant mare serum gonadothropine (PMSG) and human chorionic gonadotropine (HCG) to equate oestrus cycle. Female *Mus musculus* was injected by 5 IU PMSG intra peritoneal, after 48 hours they were injected again by HCG 5 IU intra peritoneal. After that, female Mus musculus were mated by male Mus musculus 1:1.

Tomorow morning after mating, female and male *Mus musculus* were seperated. Female *Mus musculus* were examined if they had positive vaginal plug or not. Pregnant *Mus musculus* were who had positive vaginal plug, and randomize into 7 groups (3 pregnant *Mus musculus*/group).

The location was in Laboratory of Embriology, Faculty of Veterinery, Airlangga University. This research consisted of 7 groups: K0 (control, no injection of anti QA2), K1 (anti QA2 10 ng), K2 (anti QA2 20 ng), K3 (anti QA2 30 ng), K4 (anti QA2 40 ng), K5 (anti QA2 50 ng), and K6 (anti QA2 60 ng).

K1 was injected by anti QA2 10 ng (0,1 ml) intra peritoneal in the first day of pregnant, and examined in the second day of pregnant. K2 was injected by anti QA2 10 ng (0,1 ml) intraperitoneal in the first and second day of pregnant, and examined in the third day of pregnant. K3 was injected by anti QA2 10 ng (0,1 ml) intraperitoneal in the first, second, and third day of pregnant, and examined in the fourth day of pregnant. K4 was injected by anti QA2 10 ng (0,1 ml) intra peritoneal in the first, second, third, and fourth day of pregnant, and examined in the fifth of pregnant. K5 was injected by anti QA2 10 ng (0,1 ml) intra peritoneal in the first, second, third, fourth, and fifth day of pregnant, and examined in the sixth day of pregnant. K6 was injected by anti QA2 10 ng (0,1 ml) intra peritoneal in the first, second, third, fourth, fifth, and sixth day of pregnant, and examined in the seventh day of pregnant.

Urine of *Mus musculus* was tooken in the morning and was examined for protein, creatinin, and UACR to analyze endothelial dysfunction and low renal function.

3. RESULTS

3.1. Urine Protein Level

Table 1. Data of urine protein level in all groups of *Mus musculus* with statistic results

GROUP	MEAN	SD	p VALUE	αVALUE
K0	0,633 [ad]	0,236		
K1	0,01 [b]	0,000	p=0,001	0,05
K2	0,01 [b]	0,000	(Kruskal	
K3	1,333 [ac]	0,392	Wallis	
K4	1,878 [c]	1,168	Test)	
K5	0,835 [ac]	0,147		
K6	0,623 [de]	0,092		

Notes: p value < 0,05 means there is significant differences. In each group, if there was mean that had different letter code, it was significant different. But if it had same letter code, it was not significant differences. (K0: without anti QA2, K1: anti QA2 10 ng, K2: anti QA2 20 ng, K3: anti QA2 30 ng, K4: anti QA2 40 ng, K5: anti QA2 50 ng, K6: anti QA2 60 ng).

Urine protein level started to increase in K3 (anti QA2 30 ng). Normality test with Shapiro Wilk α = 0,05 showed that not all of groups had normal distribution. So, it was continued by Kruskall Wallis test. The result of Kruskal Wallis test showed there was significant differences among all groups (Table 1). To know the different result between 2 groups, the analyze was used U Mann Whitney test α = 0,05.

3.2. Urine Creatinine Level

Table 2. Data of urine protein level in all groups of Mus musculus with statistic results

GROUP	MEAN	SD	p VALUE	αVALUE
K0	0,054	0,012		
K1	0,042	0,012	p=0,127	0,05
K2	0,040	0,015	(Kruskal	
K3	0,040	0,018	Wallis	
K4	0,020	0,005	Test)	
K5	0,070	0,012		
K6	0,058	0,014		

Notes: p value > 0,05 means there was no significant differences (K0: without anti QA2, K1: anti QA2 10 ng, K2: anti QA2 20 ng, K3: anti QA2 30 ng, K4: anti QA2 40 ng, K5: anti QA2 50 ng, K6: anti QA2 60 ng).

The highest level of urine creatinine was K5 (anti QA2 50 ng). The results of normality test showed that not all groups had normal distribution, so the analyze was continued by Kruskall Wallis test. The results were there was no significant differences among all groups (Table 2).

3.3. Urine UACR Level

Table 3. Data of urine UACR level in all groups of Mus musculus with statistic results

GROUP	MEAN	SD	p VALUE	αVALUE
K0	0,213[a]	0,102		
K1	0,287[a]	0,148	p=0,003	0,05
K2	39,433[b]	1,616	(Kruskal	
K3	57,008[b]	2,094	Wallis	
K4	50,100[b]	2,428	Test)	
K5	9,467[c]	2,793		
K6	18,850[c]	1,034		

Notes: p value <0,05 means there is significant differences. In each group, if there was mean that had different letter code, it was significant different. But if it had same letter code, it was not significant differences. (K0: without anti QA2, K1: anti QA2 10 ng, K2: anti QA2 20 ng, K3: anti QA2 30 ng, K4: anti QA2 40 ng, K5: anti QA2 50 ng, K6: anti QA2 60 ng).

Normality test with Shapiro Wilk α = 0,05 showed normal distribution in all groups but not homogen. So it was continued by Kruskall Wallis Test. The results were in Table 3. It showed that p value < 0,05, there was significant differences among all groups.

4. DISCUSSION

4.1 Urine Protein Level

Dose of anti QA2 that could increase of urine protein started from 30 ng. Urine protein happened as clinical manifestation of endothelial dysfunction process. If it hapened in pregnant condition, it could cause preeclampsia. Preeclampsia is complication in pregnant that consists of hypertension and urine protein. One of preeclampsia's patogenesis is endothelial dysfunction. Endothel is cell layer on the vascular wall that leads to lumen. Endothel functions were regulating vascular tonus, fibrinolysis system, vascular growth, and preventing trombosis (Dharma et. al., 2005).

Endothelial dysfunction happens because of oxidative stress, inflammation, and hypercholesterolemia. Oxidative stress and inflammation are basic mechanisms of preeclampsia. Endhotelial dysfunction causes disbalance of vasoactive compounds that make hypertension. Endothelial dysfunction also causes the increasing of vascular permeability, so it affects excretion of protein in urine (Dharma et. al., 2005).

Proteinuria is laboratory indicator that shows early process of low renal function that still happens. Urine protein level is an early indicator for glomerulus disturbances (Fox et. al., 2013). In this research, urine protein was found. This results showed that injection of anti QA2 since dose of 30 ng could block placental QA2 expression. The block of placenta QA2 expression made body gave inflammation response that triggered endothelial dysfunction. One of clinical manifestations of endothelial dysfunction was detected by urine protein in pregnant *Mus musculus*.

4.2. Urine Creatinine Level

The highest level of urine creatinine in this research was in K5 (anti QA2 50 ng), but Kruskal Wallis test showed there was no significant differences among all groups. It meaned that dose of anti QA that was given to *Mus musculus* could not increase significant creatinine level. The asumption was dose of anti QA that was given to *Mus musculus* had not made low renal function yet in pregnant *Mus musculus*.

This result was almost the same with Lubis et. al. (2017) that showed there was no significant differences of creatinine level in preeclampsia and normal pregnant. It was caused by process of preeclampsia's mechanism to reach low renal function still needed some processes.

Preeclampsia women who suffered endothelial dysfunction will decrease perfusion to many organs include renal. If the perfusion still decreases, it will damage renal especially in glomerulus as the location of creatinine filtration. This damage could increase creatinine level. If creatinine level had not increased yet, so the endothelial dysfunction in *Mus musculus* had not damaged glomerulus yet.

Creatinine is indicator of low renal function. It was the result of creatine dan phosphocreatine metabolisms. Creatinine is filtrated in renal glomerulus and reabsoebed in renal tubuly. In creatinine formation, there is no reuptake mechanism in our body. So creatinine can be excreted through renal (Alfonso et. al., 2016).

Dose of anti QA2 in this research could increase urine protein but could not increase urine creatinine yet, so the asumption was dose of anti QA that was given had not damage renal glomerulus yet.

4.3. Urine UACR Level

UACR is included in chronic renal disease. This ratio depends on albumine and creatinine level in urine. Table 3 showed that there was significant different of UACR among all groups. It was caused by significant different of urine protein although creatinine urine was not significant. This significant value showed that the ratio of urine protein dan creatinine in the early process of low renal function.

This results about UACR were simalr to Sogani et. al. (2014). That research concluded that UACR and serum uric acid levels as the prediction of proteinuria in new onset hypertention and uric acid in women with preeclampsia. UACR and serum uric acid progressed from mild to severe condition.

UACR can asses renal diseases. UACR level can also show the screening of microalbuminuria as the predictor of cardiovascukar/renal diseases. Microalbuminuria is defined as UACR > 2,5 mg/mmol in men and > 3,5 mg/mmol in women (Fung et. al., 2017).

UACR level showed that endothelial dysfunction that happened in pregnant Mus musculus could start low renal function. So, we must be aware to preeclampsia because the mechanism could cause low renal function. It needed early and effective prevention and treatment in preeclampsia.

5. CONCLUSIONS

The conclusions of this research were:

1. There was significant differences of urine protein level in pregnant *Mus musculus* that was injected by anti QA as endothelial dysfunction model that induced preeclampsia.
2. There was no significant differences of urine creatinine level in pregnant *Mus musculus* that was injected by anti QA as endothelial dysfunction model that induced preeclampsia.
3. There was significant differences of urine UACR level in pregnant Mus musculus that was injected by anti QA as endothelial dysfunction model that induced preeclampsia.

6. SUGGESTIONS

Preeclampsia must be detected and prevented as soos as possible and must be treated with effective treatment as soon as possible, to prevent mechanism of endothelial dysfuncyion in preeclampsia that can cause low renal function.

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