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Anti-Qa2 Animal Models for Preeclampsia Preclinical Studies: A Pathological Elevation of Blood Pressure and Proteinuria

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Original article

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Preeclampsia is the worldwide leading cause of fetomaternal morbidity and mortality indicated by increased blood pressure and proteinuria as major clinical manifestations. Although it begins with the formation of poor placentation and endothelial dysfunction, the prevention and treatment of preeclampsia still requires extensive research. We hypothesized that injections of anti-Qa2 administered during early mouse pregnancy can serve as an animal model for preeclampsia research and show the elevation of blood pressure and proteinuria associated with preeclampsia. A randomized experimental study (pretest-posttest control group design) used twenty pregnant BALB/c mice, in which the treatment group (n=10) was injected intraperitoneally with anti-Qa2 on the first through the fourth day of gestation. In this study, Qa-2 acted as a HLA-G homolog and the injection of anti-Qa2 in the first trimester led to inadequate placentation as reflected in human preeclampsia, increased systolic and diastolic blood pressure, proteinuria, placental TNFα expressions, sFlt-1 serum levels and the restriction of fetal growth with a decrease in fetal weight and length as well as placental weight ($p<0.05$). The findings suggest that the injection of anti-Qa2 in mice can be used as a validated and comprehensive preeclampsia animal model and can be used to investigate potential therapeutic interventions for preeclampsia treatment or prevention.

Key words: Preeclampsia, anti-Qa2, *Mus musculus*, animal model.

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Preeclampsia presents in 2%-8% of pregnancies and is one of the world's leading causes of perinatal mortality and morbidity (WHO 2011). Although clinical manifestations have appeared in late pregnancy, POWE *et al.* (2011) found that pathophysiological changes in the form of early poor placentation in preeclampsia cause the forming of a non-dilated spiral artery with a tunica media layer and a lumen diameter which remained unchanged. However, the onset occurs in the latter half of pregnancy as stated by STEEGERS *et al.* (2010) which is indicated by hypertension, a persistent diastolic blood pressure of ≥90 mm Hg and the emergence of proteinuria of ≥0.3 g/24 h. The pathogenesis of preeclampsia is partially understood as many hypotheses have been developed in terms of causal factors: placental vascularity abnormalities, placental ischemia, free radicals, endothelial dysfunction, immunologic intolerance between mother and fetus, abnormal genetic cardiovascular adaptation, nutritional deficiency, and inflammatory theories (CUNNINGHAM *et al.* 2014; HLADUNEWICH *et al.* 2007; JIM *et al.* 2010). Based on the several hypotheses above, AHMED and RAMMA (2015) referred to preeclampsia as *the disease of theories* and the theory underlying preeclampsia is inadequate placentation (STEEGERS *et al.* 2010).

In developing countries, almost ten percent of maternal deaths are related to gestational hypertension with preeclampsia-eclampsia as the most

common cause. The World Health Organization (WHO) in 2011 stated that, optimizing health care to prevent and treat this complication is a necessary step toward development goals. So far, scientists have been unsuccessful in their efforts to better understand preeclampsia and to prevent and reduce the occurrence of morbidity and mortality caused by preeclampsia in humans (MCCARTHY *et al.* 2011). Furthermore, recent studies about preeclampsia prevention and treatment are still using animal models to understand how pharmacology and therapy intervention affect the pathophysiology mechanism of preeclampsia before clinical trials.

MCCARTHY *et al.* (2011) suggested that an ideal animal model has to represent the pathology of preeclampsia in pregnant humans initiated with impaired trophoblast invasion. Furthermore, it is characterized by specific maternal syndromes: hypertension, excess protein excretion in urine, endothelial dysfunction, angiogenesis imbalance and precise fetal results. Recent preeclampsia animal model methods in previous studies and reviews are: the reduced uterine perfusion pressure model by FUSHIMA *et al.* (2016), nitric oxide abnormalities with knockout mice by HUANG *et al.* (1993), mice with deficiency in catechol-O-methyltransferase and 2-methoxyoestradiol by KANASAKI *et al.* (2008), impaired renin-angiotensin-aldosteron system with transgenic mice by BROSNIHAN *et al.* (2010) or with an exogenous injection of AT1-AA (angiotensin type II receptor autoantibodies) by ZHOU *et al.* (2008), BPH/5 hypertensive mouse strain by DOKRAS *et al.* (2006), angiogenesis imbalance with a sFlt-1 (soluble Fms-like tyrosine kinase-1) injection reviewed by MAYNARD & KARUMANCHI (2011), immunological models with a TNFα injection by LAMARCA *et al.* (2005), insulin resistance models by PODJARNY *et al.* (1998), the ROS (reactive oxygen species) model by FAAS *et al.* (1994) and other models such as chronic stress by TAKIUTI *et al.* (2002).

Each method has its advantages, disadvantages and limitations. All of the methods meet the expected criteria necessary to be comprehensive preeclampsia animal models and show several of the manifestations of preeclampsia in pregnant humans. However, MCCARTHY *et al.* (2011) has reviewed and stated that generally, the methods using exogenous injection treatment were administered after the first trimester when the critical time of placentation had already ended. After this vital time, models using gene manipulation were less useful as a means to study trophoblast invasion and vascular remodeling in preeclampsia. Therefore, we tried to explore the alternative use of a preeclampsia model using anti-Qa2 injections, considering that Qa2 is the functional homolog of human HLA-G. HLA-G is an important fetomaternal tolerance protein that serves as a ligand for killer inhibitory receptors in maternal natural killer cells which then inhibit a maternal immune response against the fetus. SULISTYOWATI *et al.* (2017) have shown that anti-Qa2 can be used for preeclampsia models as indicated by the decrease of PIGF (placental growth factor), VEGF and the increase of the anti angiogenic factor sFlt-1, glomerular endotheliosis and fetal growth restriction in their results. In our study, we learned how anti-Qa2's interruption of placentation and increase of blood pressure as well as urine protein excretion data can support the previous study and be a comprehensive model to investigate potential therapies for preeclampsia.

Materials and Methods

Animal handling and experimental procedures in this study followed recommendations in the Guide for the Care and Use of Laboratory Animals (ILAR 2011). All protocols were approved by the Animal Care and Use Committee (ACUC), Faculty of Veterinary Medicine, Airlangga University No. 2.KE.001.01.2018.

Experimental Design and Sampling

This was an experimental study using a randomized pretest-posttest control group design and twenty female mice (*Mus musculus*) from The Integrated Research and Testing Laboratory, Gadjah Mada University, Indonesia. The experiment was carried out in the Faculty of Veterinary Medicine, Airlangga University, Indonesia, where mice were adapted to the laboratory conditions and procedures for one week before onset of the experiment. The mice were healthy BALB/c mice aged 3 months with a 20-25 g body weight, housed in plastic cages with a wooden chip bedding in a temperature-controlled room (23°C). They were given food and water ad libitum, and exposed to a 12-hour light-dark cycle (ECKEL-MAHAN & SASSONE-CORSI 2015). Following STEEL *et al.* (1997) a sample group of 20 mice was randomly divided into 2 groups on the first day of gestation: a control and anti-Qa2 injection group. The samples' treatment was performed in the laboratory under controlled and constant conditions and they were observed if there was a change in body weight during the study.

Mating Procedure

All of the mice were prepared for mating with oestrus cycle synchronization following the procedures of THE PRACTICE COMMITTEE OF THE AMERICAN SOCIETY FOR REPRODUCTIVE MEDICINE, 2008. This method is used to induce oestrus,

ovulation and coitus in female mice and allows one to observe and determine the time required for entering oestrus, including the successive meiotic stages from prophase I through the emission of the first polar body at metaphase II, as well as ovulation and implantation. All mice were injected with 5 IU of Pregnant Mare's Serum Gonadotropin (PMSG PG 600, MSD Animal Health, Intervet Inc.), followed by human Chorionic Gonadotropin (hCG Chorulon, MSD Animal Health, Intervet Inc.) forty-eight hours later. The mice were then mated one-on-one with mature male mice (aged 7 months, weighing ca. 40 g). The presence of a copulatory plug (sperm plug) seventeen hours later was considered as day 0 of gestation.

Anti-Qa2 Injection

SONES and DAVISSON (2016) have reviewed trimester demarcations in mouse pregnancy. Mice have a relatively short length of gestation (~20 days) compared to humans. However, the important 1st-trimester early pregnancy milestones leading up to placental formation occur over the first half of the gestation period in mice. In this study, we injected intraperitoneally the treatment group with 10 ng of anti-Qa2 on the 1st through the 4th day of gestation, knowing that implantation occurs in approximately the first quarter of murine pregnancy. Intraperitoneal injection in upper right abdomen was done with a slope of 45° and 0.5 cm deep with one cc syringe and a 27G needle. SONES and DAVISSON's (2016) study showed that an intravenous injection of anti-Qa2 led to an increase of the heat shock protein (HSP-70) and the vascular cell adhesion molecule-1 (VCAM-1) in preeclampsia, in which Qa2 was normally expressed on the fifth day of gestation in mouse pregnancy. We used intraperitoneal injection (IP) rather than intravenous injection (IV) because it is similarly efficient in terms of bioavailability, without the impracticality and risk of perivascular trauma that comes with using the IV method. TURNER *et al* (2011) also stated that routine intravenous injections have the potential to cause severe complications, including blindness, cerebrovascular stroke, permanent motor deficits, and limb gangrene. Most previous preeclampsia animal model studies also used intraperitoneal injection, such as the studies using a TNF-α injection by WICAKSONO *et al.* (2015) and a human preeclampsia serum injection by KALKUNTE *et al.* (2010).

Measurement of results

Blood pressure

The samples' systolic blood pressure was measured using the mouse blood pressure system (MRBP-MMC, IITC Life Science Inc, CA, USA) using the tail-cuff method on day 0 and 15 of gestation. Diastolic blood pressure was calculated using the software, MRBP Monitor Version 1.59 (MRBP-MMC, IITC Life Science Inc, CA, USA). The use of non-invasive blood pressure assessment is reliable, accurate, easier than and clinically similar to the direct blood method without requiring the surgery needed for arterial catheterization. In addition, CROY *et al.* (2015) state that non-invasive blood pressure assessment also provides on point single data when monitoring the blood pressure of mice. This method enables blood pressure (BP) and blood volume change to be measured in mice by using a specialized volume pressure recording (VPR) sensor placed over the animal's tail (WANG *et al.* 2017). Before taking the measurements we used a warming plate to stabilize the samples' body temperature and prepared them through one week of training to avoid inappropriate data due to stress.

Protein urinalysis

24-hour urine was collected on the 15th day of gestation or in the second half of pregnancy (>20 weeks in human pregnancy during which the clinical manifestations of preeclampsia occur). In order to avoid inaccurate data, we also collected 24-hour urine on day 0 of gestation to compare both groups. Mouse urine was collected using a single cage design based on the modified model from KURIEN *et al.* (2004) consisting of a polyethylene funnel (22.5 cm in diameter) with a nylon screen inserted into the funnel (14 mesh/in. cut to a 12.5 cm diameter). A stainless steel screen (4 mesh/ 2.5 cm cut to a 11.25 cm diameter) was placed over the upturned edges of the nylon screen and fastened to the funnel with a wire. The mouse was placed in the funnel and a plastic lid with ventilation holes was mounted over it. The funnel was placed in an appropriate holder with a tube fastened underneath to collect urine. On the days of urine collection, the mice were given water *ad libitum*, no food was given to avoid urine contamination by feces.

The collected urine was tested using a semi-automated urine test strip analyzer, Cobas u 411 Urine Analyzer (Roche Diagnostics Ltd., Switzerland) and Miditron Junior II Combur 10 Test strips (ref. 11379208). A semi-quantitative method was used because the minimum criteria for proteinuria in preeclampsia according to the Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy by ROCELLA (2000), is ≥300 mg/day, and the measurement of urine creatinine concentrations at every prenatal visit is unrealistic due to medical costs. This is why the most common

method of measuring the protein concentration of 24-hour urine used in outpatient clinics is the dipstick test. However, in order to avoid false positives which were commonly found on ±1 results, previous study by MAKIHARA *et al.* (2010) used a ≥2+ cut off and the specific gravity as additional determination of pathological proteinuria. In addition, another study by SYUHADA *et al.* (2012) reported a positive correlation between urine's albumin:creatinin ratio (uACR) and the dipstick method, so this simple method can still be used.

Sample termination

All samples were terminated on the 16th day of gestation as corresponding to the onset of the third trimester of human pregnancy, when preeclampsia usually manifests (SULISTYOWATI *et al.* 2017). The surgical procedure was performed under general anesthesia using an intramuscular injection of ketamine. Then, numbers of developed and resorbed fetuses were counted, all fetuses (those developed and resorbed) were measured and weighed, and placental weight was also recorded.

Immunohistochemistry for placental TNFα examination

Samples of the placenta were fixed in 7% buffered formalin while the paraffin sections were prepared. The expression of TNFα was demonstrated using anti-mouse TNFα antibodies (BioLegend, USA, catalog number 506249) at a 1:100 dilution. The reaction product was visualized using a kit of reagents and diaminobenzidine (DAB). The sections were pre-exposed to the procedure of boiling in a microwave oven with antigen retrieval solution, in order to unblock antigenic determinants. In order to examine the placental TNFα, we used a modified semi-quantitative IRS scale of Remmele based on the work of NOWAK *et al.* (2007). This method relied on both the percentage of positive cells and the intensity of the color reaction. The final score (ranging from 0 to 12 pts) represented the product of the parameters as follows: no reaction – 0 pts (-); poor reaction – 1-2 pts (+), moderate reaction – 3-4 pts (++), intense reaction – 6-12 pts (+++).

ELISA for sFlt-1 serum levels

Mouse blood was taken directly from the right heart, then centrifuged at 6000 rpm for 10 minutes. Serum was taken and measured for mouse sFlt-1 concentrations using the sFlt-1 ELISA kit (Bioassay Technology Laboratory, China, catalog number E0611Mo) which uses a quantitative sandwich enzyme immunoassay technique and was performed according to the manufacturer's protocol. The optical density of each well was measured by using an ELISA reader set to 450 nm.

Statistical analysis

Blood pressure, placental TNFα expressions and sFlt-1 serum levels were analyzed using an independent t-test and fetal growth restriction was measured using a paired t-test with a 95% confidence interval as the data were normally distributed. The proteinuria data was converted to a nominal scale and analyzed using a χ^2 test based on the methods of MAKIHARA *et al.* (2010). Statistical analyses were performed using SPSS version 22.0 for Windows (IBM SPSS, IBM Corp., Armonk, N.Y., USA) with $p \leq 0.05$ considered as significant difference threshold.

Results

The effect of Anti-Qa2 on blood pressure

In this study, we injected the treatment group with anti-Qa2 and measured their systolic and diastolic blood pressure. The decrease in peripheral vascular resistance and arterial blood pressure, as adaptation during normal pregnancy, are usually absent or reversed in preeclampsia. LEVINE *et al.* (2006) identify preeclampsia by the increased systemic vascular resistance and lower cardiac output that comes as a result of the placental ischemia and dysfunction. As shown in Figure 1, there were no differences in systolic and diastolic blood pressure between both groups on day 0 of gestation (p=0.738 and p=0.224, respectively). When measured on the 15th day of gestation, there was a significant increase in both the systolic (178.9±18.89 vs. 109.5±9.56; p=0.000) and diastolic (162.30±23.38 vs. 70.10±13.30; p=0.000) blood pressure of the treatment group compared to the control group (Fig. 2).

The effect of Anti-Qa2 on proteinuria

We used a semi-quantitative method of protein urinalysis in which a highly buffered indicator changes its color in the presence of proteins (anions) as a result of the indicator releasing hydrogen ions to the protein. As described by the manufacturer, the category of proteinuria results from dipsticks scanned in the urine analyzer is –, 1+, 2+, 3+ as negative, 15-30 (±25) mg/dl, 31-100 (±75) mg/dl and 101-5000 mg/dl, respectively. The dipstick test results were classified into 3 groups: –, 1+, and ≥2+ which were then converted to 1, 2, and 3 respectively, for statistical analyses using a χ^2 test for both day 0 and day 15 of gestation, shown in Table 1.

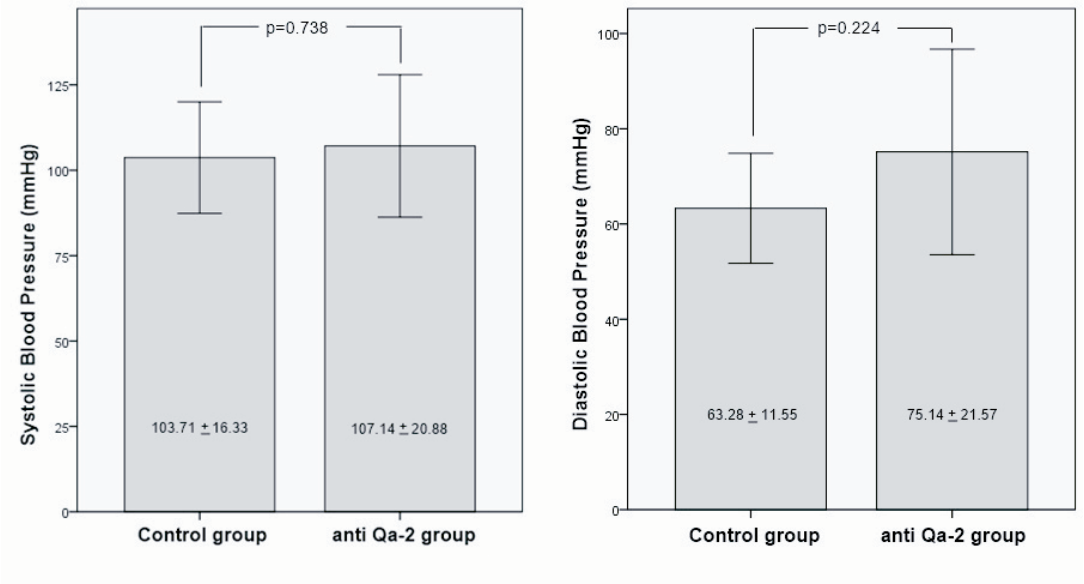

Fig. 1. Systolic and diastolic blood pressure (mean ±SD) on day 0 of gestation. There was no significant difference between the treatment and control groups (p>0.05).

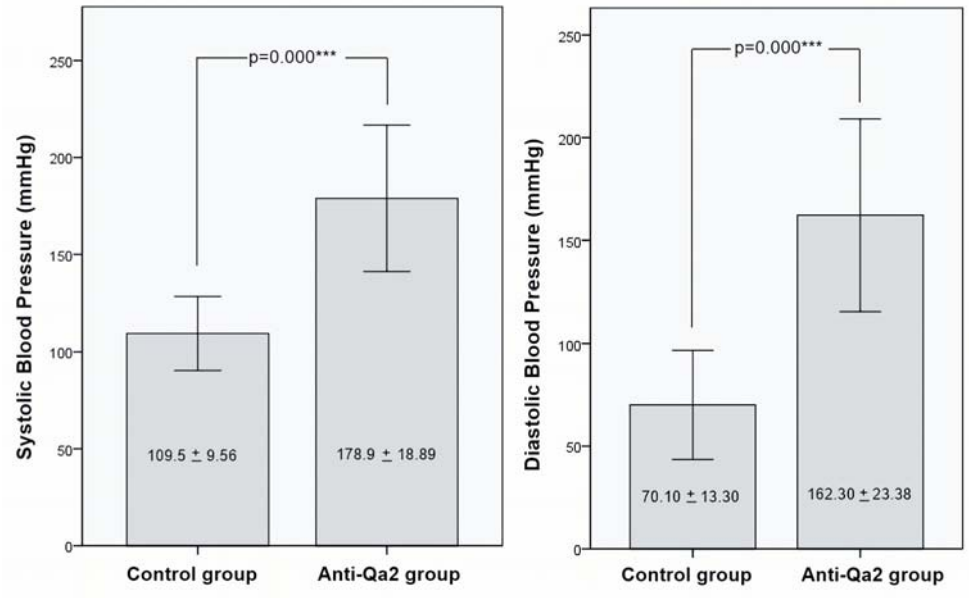

Fig. 2. Systolic and diastolic blood pressure (mean ±SD) on the 15th day of gestation. Anti-Qa2 treatment group had higher systolic and diastolic blood pressure than the control group (***highly significant difference at p<0.001)

Table 1 shows that there were no significant differences between the two groups on day 0 of gestation (p=0.264). On the 15th day of gestation, the control group had a 60% sample in the negative category of proteinuria, a 40% sample in 1+ and nothing in 2+ category. On the other hand, the treatment group had a 10% sample in the negative category, a 20% sample in 1+ and a 70% sample in 2+. It was expected that the treatment group would have most results in the highest category. The treatment group had proteinuria as a 2+ category or ±75 mg/dl in 7 samples, while the control group had nothing in this category. This resulted in a statistically significant p-value (p<0.05). As proteinuria is an important clinical diagnostic test for preeclampsia, it has been agreed that the minimum result fell to the 1+ category or 300 mg or 30 mg/dl in 24-hour urine. In our study some data also showed positive or 1+ in the control group and a negative category in the treatment group. In previous studies, this method had a 56-80% sensitivity and a 67-92% specificity (CHOTAYAPORN *et al.*

Table 1

Semi-quantitative dipstick results of proteinuria in 20 samples. The anti-Qa2 group shows more samples in the 2+ category

Dipstick test results	Control group		Anti Qa-2 group		Total Day-15
	Day-0 (n=10)	Day-15 (n=10)	Day-0 (n=10)	Day-15 (n=10)	
-	9	6	7	1	7
1+	1	4	3	2	6
2+	0	0	0[1]	7**	7
Total	10	10	10	10	20

[1] no significant differences between both groups on day 0 of gestation (p=0.264)
**statistically significant result on the 15th day of gestation (p=0.004).

2011) and there were 92% of cases which indicated 300 mg of proteinuria in 1+ dipsticks, otherwise the negative of trace results (<150 mg proteinuria) had a negative prediction in 34% of hypertensive women (CUNNINGHAM *et al.* 2014). When comparing the pH and specific gravity (SG) tests, no samples had too acidic of a pH (<4.5) or too alkaline pH (>9) and no sample had a SG<1.010 or SG>1.030, indicating that we avoided showing false positive or false negative data.

The effect of Anti-Qa2 on placental TNFα expression

Since inflammation is a preeclampsia pathway theory, we evaluated placental TNFα with an immunohistochemical method to investigate the etiology of preeclampsia in the control group (n=10) and the treatment group (n=10). The differences between both groups were shown in Figure 3 then inflammatory degrees were scored with a modified Remmele method and resulted in immune-reactive scores (IRS) for 5 field-of-views as shown in Figure 4. We used an independent t-test analysis ($p<0.05$) on the placental TNFα immuno reactive score which resulted in a significantly increased expression in the treatment group compared with the control group (7.04±1.87 vs. 10.24±2.15; p=0.040). Figure 3 B shows the higher expression of placental TNFα in the treatment group indicated in brown as chromogen expression.

The effect of Anti-Qa2 on serum sFlt-1 levels

The etiology of preeclampsia involves adapted vasculogenesis and angiogenesis. The imbalance between angiogenic and antiangiogenic factors is known as one of the predictors of preeclampsia. In this study we measured the sFlt-1 concentrations as the antiangiogenic factor in mouse serum the results of which are illustrated in Fig. 5 which shows the difference between the serum concentrations of sFlt-1 in both the control and treatment group (448.86±64.86 ng/ml vs. 560.14±46.58 ng/ml, p=0.005). This data are supported by elevated blood pressure and proteinuria as main clinical manifestations.

The effect of Anti-Qa2 on fetal growth restriction

Table 2 presents the fetal growth in each group and the number of fetuses developed and resorbed. Fetus weight, length and placental weight were statistically different with $p<0.05$. The treatment group had less developed fetuses than the control group. Anti-Qa2 also increased the number of resorbed fetuses and reduced other characteristics such as fetal weight, fetal length and placental

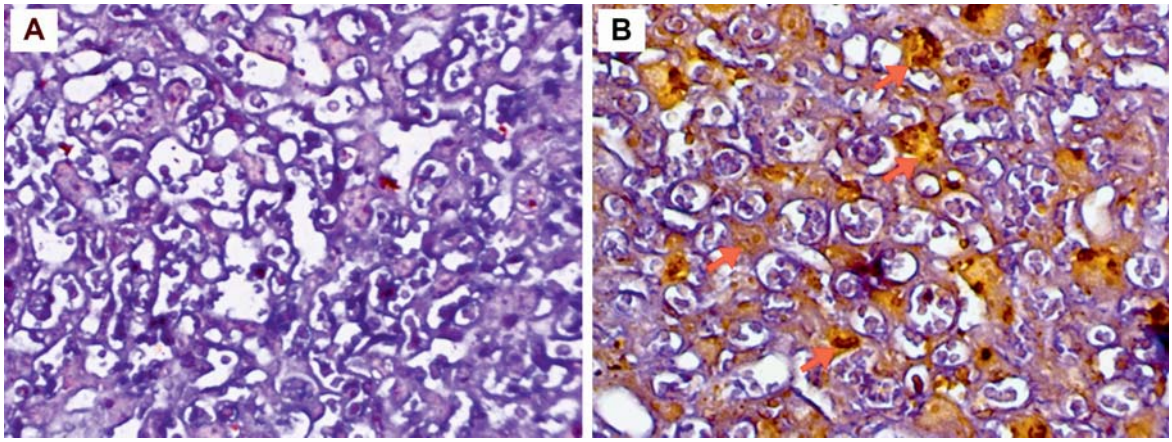

Fig. 3. Immunohistochemical analysis of samples' placental TNFα expression: A – control group and B – anti-Qa2 treatment group; the arrows indicate placental TNFα expression in trophoblast cells indicated by a brown chromogenic color. Magnification 400x.

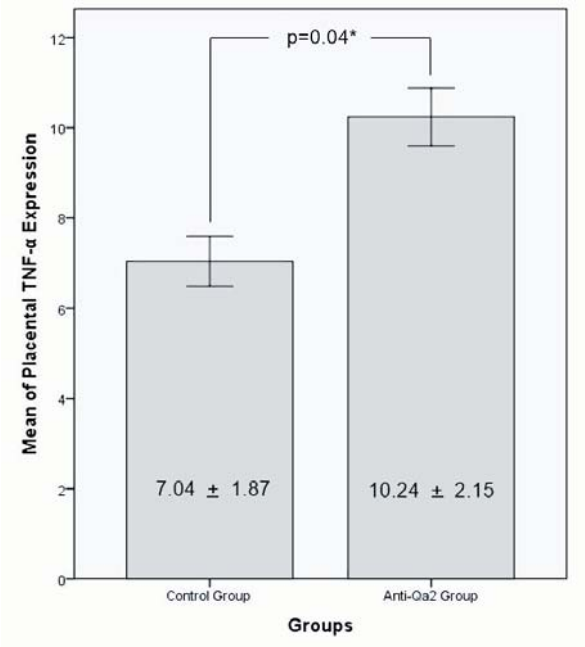

Fig. 4. Immuno-reactive score results of placental TNFα expression (mean ±SD). The anti-Qa2 treatment group had a significant increase in the expression of placental TNFα (*p<0.05).

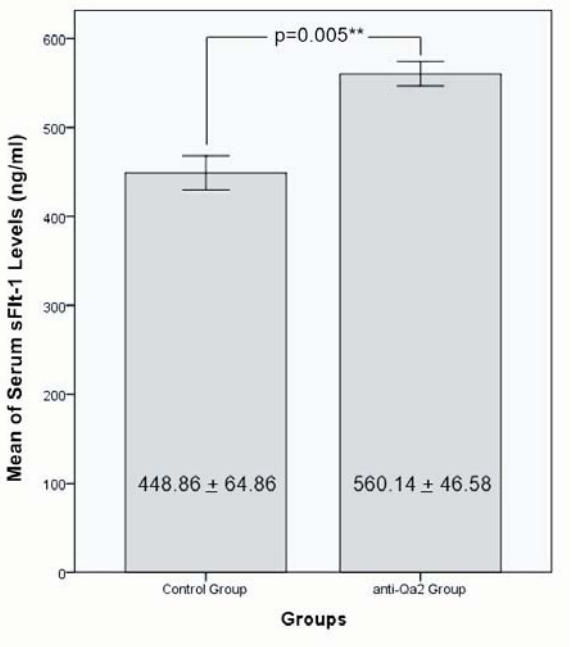

Fig. 5. Serum concentrations of sFlt-1 in BALB/c mice (mean ±SD). The anti-Qa2 treatment group showed an increase in sFlt-1 production (**p<0.01).

Table 2

The fetal growth in each group: the number of developed fetuses and resorbed fetuses; fetus weight, length and placental weight differ significantly (data shown as mean ±SD)

Comparison of variable	Control group (n=10)	Anti Qa-2 group (n=10)	p
Number of developed fetuses	86	78	–
Number of resorbed fetuses	1	14	–
Fetal weight (g)	0.792±0.322	0.233±0.179	0.000***
Fetal length (mm)	23.038±6.062	11.129±4.641	0.000***
Placental weight (g)	0.150±0.034	0.085±0.059	0.000***

***highly significant difference at p<0.001

weight. Table 2 shows that anti-Qa2 restricted growth as fetuses from the control group (0.792 g) were bigger than those of the treatment group (0.233 g) with a p-value=0.000. In this study, we assumed that fetal growth restriction was symmetrical, indicated by shorter fetal length in the treatment group which was 11.129 mm compared to the control group which was 23.038 mm (p=0.000). An injection of anti-Qa2 resulted in a significant (p=0.000) restriction of intrauterine growth demonstrated by the reduced placental weight (0.085 g) of the treatment group compared to the control group (0.150 g).

Discussion

In this study, we injected healthy female BALB/c mice with anti Qa-2 during their early pregnancy period which resulted in an increase of blood pressure, proteinuria, placental TNF expression, sFlt-1 production and reduced intrauterine

growth. Previous studies using the same haplotype of mice showed that Qa-2 is a murine homolog of human leukocyte antigen-G (HLA-G) as non-classical class Ib major histocompatibility complex (MHC) located in the Q region (Q6, Q7,Q8, and Q9) (SILVA *et al.* 2017; WU *et al.* 1998). Qa-2 is considered a HLA-G homolog as both families of protein have similar characteristics, such as the presence of membrane-bound and soluble forms that arise from alternative splicing, their involvement in pre-implantation embryo development and their immunoregulatory roles (DJURISIC & HVIID 2014).

HLA-G regulation has been reported by DURMANOVA *et al.* (2013) and DJURISIC and HVIID (2014) as the pre-implantation embryonic development gene which plays an important role as a killer inhibitory receptor (KIR) ligand for maternal NK cells. The dysregulation of human leukocyte antigen (HLA)-G was found in both the placentas and maternal sera of preeclampsia patients. The beginning of a normal pregnancy involves several immunological responses including the regulation of Th1, TNFα, IFN-γ, Th2, IL-4 and IL-10 which support embryonic implantation, angiogenesis, trophoblast development and the determination of immune tolerance to an allogenic fetus. Later, a normal pregnancy has increased regulatory T cell activity and decreased natural killer (NK) cell activity (SAITO 2010). Furthermore, TANG *et al.* (2015) said that the hypermethylation of HLA-G is associated with preeclampsia, indicated by the transformation of the DNA-methylation of the HLA-G promoter region which leads to a decrease in the expression of HLA-G.

We injected the treatment group with anti Qa-2 during the early pregnancy period due to the differences between human and murine pregnancy reported by SONES and DAVISSON (2016). Mouse pregnancy is shorter (~20 days), but the first half of the gestation period is similar to the first trimester of human pregnancy. It is during this period that fertilization, implantation and decidualization occur. As mentioned before, an ideal preeclampsia animal model should represent the central processes of preeclampsia, trophoblast invasion and vascular remodeling, which occur in the first trimester of pregnancy periods (MCCARTHY *et al.* 2011). In our study, an injection of anti-Qa2 led to poor placentation and other symptoms that represent the pathogenesis of preeclampsia in the preclinical stage such as, impaired remodelling of the uterus' spiral arteries (endothelial dysfunction), an imbalance of circulating angiogenic factors causing systemic endothelial dysfunction and resulting in hypertension, proteinuria, as well as other systemic manifestations common in the clinical stage (>20-week human pregnancy or >10.5-day in murine pregnancy). In our study, the data collected showed the differences between the control and treatment group in terms of blood pressure, proteinuria and growth restriction (p=0.000). The pathological cut off for this data follows the study by ZENCLUSSEN *et al.* (2004) which found that animal models of preeclampsia and gestational hypertension had recorded blood pressures of up to 50 mmHg higher than control groups as well as the study of VENDITTI *et al.* (2014) which stated that the cut off for proteinuria is >300 mg protein or 1+ dipstick or 0.3 for the urine albumin:creatinin ratio (uACR). In our study, an injection of anti-Qa2 caused angiogenesis and vasculogenesis disruption which increased vascular resistance, resulting in the elevation of blood pressure. Another effect of anti-Qa2 was a systemic inflammation response and glomerular endotheliosis, an increase in the vascular permeability of the glomerular capillary wall, resulting in an increase of proteinuria.

In this study, we also measured placental TNFα, sFlt-1 concentrations and intrauterine growth to further investigate the potential of anti-Qa2 as an animal model of preeclampsia mechanism. We recorded an increase in all three variables in response to an injection of anti-Qa2. The dysregulation of TNFα production in preeclampsia causes an imbalance of the angiogenic factor, which disrupts placental development. This leads to a limited cytotrophoblast invasion of the arteries in the superficial decidua while myometrial segments remain narrow and undilated. In regards to this condition, MAYNARD and KARUMANCHI (2011) found that growth restriction resulting from an injection of anti-Qa2 might be caused by hypoxia and ischemia, which disrupt placental blood flow, rather than the factors occurring in preeclampsia. Despite our attempts to obtain data comparable with other studies, data differences in blood pressure, proteinuria, placental TNFα, sFlt-1 concentrations and intrauterine growth restriction may occur between our study and previous studies due to either the mice haplotype or measurement methods. For instance, measuring blood pressure with a non-invasive tail cuff could result in lower readings than studies using other methods (WANG *et al.* 2017).

Our study showed that an injection of anti Qa-2 in the first trimester of mouse pregnancy increased blood pressure and proteinuria, the main clinical manifestations of preeclampsia. These increases were then followed by the increase of inflammatory cytokines, anti-angiogenic factor production and the restriction of intrauterine growth recorded as a decrease in fetal weight, length and placental weight. On the basis of our findings, we propose a possible role underlying the Qa-2 as HLA-G homolog in pregnancy. Although this study only serves to support the data of previous studies re-

garding anti Qa-2 animal models for preeclampsia resulting in the increase of sFlt-1, VCAM-1, HSP-70 and in glomerular endotheliosis (SULISTYOWATI *et al.* 2010; SULISTYOWATI *et al.* 2017), it contributes to our overall knowledge of the use of this method in the prevention or treatment of preeclampsia, an area of study which still needs to be further investigated.

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Author Contributions

Research concept and design: E.M.H., P.L., W.; Collection and/or assembly of data: E.M.H., W.; Data analysis and interpretation: E.M.H., W.; Writing the article: E.M.H., P.L.; Critical revision of the article: E.M.H., P.L.; Final approval of article: E.M.H., P.L., W.

Conflict of Interets

The authors declare no conflict of interest.

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