Research Report

Low Class Ib (HLA-G/Qa-2) MHC Protein Expression against Hsp-70 and VCAM-1 Profile on Preeclampsia. An observation on experimental animal Mus Musculus with Endothelial Dysfunction model

Ekspresi Protein MHC Klas Ib (HLA-G/Qa-2) yang Rendah Terhadap Profil Hsp-70 dan VCAM-1 pada Preeklampsia Penelitian pada Hewan Coba Mus Musculus dengan Model Disfungsi Endotel

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Abstract

Objective: To analyze the expression of MHC class Ib Qa-2 protein (homologue to HLA-G in human), Hsp-70, and VCAM-1 in trophoblast cells of preeclampsia and control using endothelial dysfunction model on Mus Musculus.

Method: Design of study is experimental study. Pregnant mice was treated with anti-Qa-2. Hsp-70 and VCAM-1 expressions of trophoblast cells was assessed on week I, II, and III.

Result: Negative Qa-2 expression was achieved after administration of 40ng anti Qa-2 on the fifth day of pregnancy. The Hsp-70 and VCAM-1 expressions in negative Qa-2 mice (preeclampsia) was higher compared with those of positive Qa-2 mice (normal pregnancy) on week I, II and III.

Conclusion: Low Qa-2 and high of HSP-70 and VCAM-1 expression might be useful for prediction of preeclampsia or endothelial dysfunction.

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Keywords: preeclampsia, HLA-G/Qa-2, Hsp-70, VCAM-1, endothelial dysfunction

Abstrak

***Tujuan**: Untuk menganalisis ekspresi protein MHC Klas Ib Qa-2 (homolog HLA-G pada manusia), protein Hsp-70 dan protein VCAM-1 di trofoblas pada preeklampsia dan hamil normal dengan menggunakan model disfungsi endotel pada hewan coba Mus Musculus.*

***Metode**: Eksperimental dengan cara membuat mencit bunting tidak mengekspresikan Qa-2 dan menganalisis ekspresi Hsp-70 dan VCAM-1 pada trofoblasnya baik pada minggu I, II maupun III.*

***Hasil**: Qa-2 negatif terjadi pada dosis 40 ng yaitu pada kebuntingan hari ke-5. Ekspresi Hsp-70 dan VCAM-1 pada mencit bunting Qa-2 negatif (preeklampsia) lebih tinggi dibandingkan dengan ekspresinya pada mencit bunting Qa-2 positif (hamil normal) baik pada kebuntingan minggu I, II maupun III.*

***Kesimpulan**: Ekspresi HLA-G/Qa-2 yang rendah, protein Hsp-70 dan VCAM-1 yang tinggi merupakan prediktor untuk terjadinya preeklampsia (disfungsi endotel).*

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***Kata kunci**: preeklampsia, HLA-G/Qa-2, Hsp-70, VCAM-1 disfungsi endotel*

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INTRODUCTION

Preeclampsia is a disorder of pregnancy marked by hypertension and proteinuria after 20 weeks of gestation. Preeclampsia is still one of the major causes for maternal morbidity and mortality. The general prevalence of preeclampsia is 4.4 - 17.5%, with average of 4.6%.[1] In Indonesia, the incidence of preeclampsia is 3 - 10%, and contributes to 39.5% of maternal mortality in 2001, and 55.56% in 2002.[2]

Transplantation of organs were related with major histocompatibility complex (MHC), such as human leucocyte antigens (HLA). In pregnancy, there is invasive interaction between decidua and cytotrophoblast. The Human Leucocyte Antigens-G (HLA-G) have role in semiallogenic immune tolerance of the fetus that adequate HLA-G expression of the trophoblast will prevent such maternal immune response. The decidua contains Large Granular Lymphocytes (LGLs), such as Natural Killer Cell (NK cell), which will destroy any cell with inadequate HLA-G expression. HLA-G is monomorphic class Ib non-classic HLA molecule, which is able to inhibit the activity

of NK cells and the decidual LGL. Thus, HLA-G possesses the function of protecting trophoblast from the influence of maternal immune system, or cytotoxic attack.[3] During pregnancy, the trophoblast increase regulation of HLA-G expression.

An adequate HLA-G expression is needed for trophoblastic invasion into decidua and maternal vascular system. This invasive trophoblast cells might mediate changes that on increase in uterine perfusion is needed to ensure adequate blood supply during pregnancy.

Without proper invasion, the maternal artery remodelling did not occur and subsequent decrease of utero-placental supply will cause placental hypoxia, a condition which precludes preeclampsia. This is in accordance with the hypothesis that preeclampsia is caused by abnormality in utero-placental local immune recognition, between mother and fetus.[3]

Qa-2 protein, product of Ped gene in mice, is a MHC class Ib protein related to the glycophosphatidil inositol (GPI) found on the surface of T cell and preimplanted embryo in ice.[4] The Qa-2 protein manage early embryonic development in mice, and its subsequent survival. This protein is expressed on the surface of preimplanted mice embryo from two cell stage up to blastocyst stage, after which it will be expressed by inner cell mass and trophoectoderm.

The mice embryo that expresses Qa-2 have faster cell division, better survival and better birth weight compared to the control. On the contrary, embryo that does not express Qa-2, shows miscarriage, having an embryonal/fetal death, premature birth and low birth weight.[5]

Heat shock protein (Hsp) is known as protecting cells against cytotoxic disturbance. Hsp is a group of inducible protein, in which, some are expressed constantly, and increased as a respond to stress, while others are expressed later. The permanently expressed proteins have role as a messenger for other cell production and binds polypeptides that prevent premature folding, and as a translocation factor for proteins into the organels. Induced stress proteins can protect cells from stress-induced damage by preventing protein denaturation and or by fixing the damage.

Hsp overexpression has an important role in cell protection during physiologic stress against apoptosis. In preeclampsia, it is possible that the placenta system experience an overwhelming stress, and this may be the reason for increased in Hsp production.

Xu (*et al*) wrote that Hsp may play a role in the repair of denaturated protein caused by oxidative stress and in the folding of new polypeptide chain, thus the Hsp-70 is important for keeping homeostasis during oxidative stress. Oxidant free radical may trigger the increase of Hsp-70 in order to protect cells from oxidative stress and apoptosis in preeclampsia. It is concluded that the increase of Hsp-70 is related to the placenta of preeclamptic women.

Preeclampsia was related with the increase of soluble VCAM, e-selectin, and ICAM-1 in the maternal serum. This increase may constitute a beginning. The increase in soluble cell adhesion molecule reflects the increase of various cell adhesion molecules in endothelial cell, and explains the leucocyte activation in preeclampsia.

Research using human trophoblastic tissue of first trimester is not common, as it may cause abortion and is not ethical. Researchers then use laboratory animal to study preeclampsia at early gestation to assess any endothelial damage or dysfunction in the placental vasculature.[7] Even though there were dissimilarity between preeclampsia in la-boratory animal and in human, we can use laboratory animal as comparison.

The objective of this research is to assess Qa-2 (homolog to human HLA-G), Hsp-70 and VCAM-1 protein expression in trophoblast of preeclamptic and control using laboratory animal Mus Musculus with endothelial dysfunction model.

METHOD

This study was performed in the animal pen laboratory of Airlangga University Veterinary Medicine Faculty to obtain pregnant mice, anti-Qa-2 treatment and maintain their pregnancy to 1st, 2nd and 3rd week of gestation. Immunofluorescence and immunohistochemocal studies were performed in Pathology Laboratory of Airlangga University, Faculty of Veterinary Medicine. This study was conducted from August to September 2009. The choice to use Mus Musculus mice was based on genetic similarity with human and adaptability in laboratory environment.[8] The number of sample was 120 (60 mice each for the first and second stage) based on the replication formula of Steel and Torrie.[9] The first stage in this study is to mate the mice, while the second stage is to administer of anti Qa-2.

First Stage Research

Mice impregnantion

We synchronized the mice estrus by injecting 5 IU Pregnant More Serum Gonadotrophin (PMSG) hormone, followed by 5 IU Human Chorionic Gonadotrophin (hCG) injection 48 hours later, to 3 - 4 months old female mice. Female mice are then mated using monomating technique. They were put alternately in a cage that contained one 7 months old male mice weighed 60 grams. Diagnosis of pregnancy was established 17 hours after mating with the presence of copulatory plug.

Treatment of anti Qa-2

After diagnosis of pregnancy, they were separated into 6 groups (each contains 10 mice). Group I (control) did not receive anti Qa-2 treatment. Group II received 10 ng of anti Qa-2 on day 1 and Qa-2 was assessed on day 2 of pregnancy. Group III received 10 ng of anti Qa-2 on day 1 and 2 and Qa-2 was assessed on day 3 of pregnancy. Group IV received 10 ng of anti Qa-2 from day 1 to 3 and Qa-2 was assessed on day 4 of pregnancy. Group V received 10 ng of anti Qa-2 from day 1 to 4 and Qa-2 was assessed on day 5 of pregnancy. Group VI received 10 ng of anti Qa-2 from day 1 to 5 and Qa-2 was assessed on day 6 of pregnancy.

Qa-2 analysis using immunoflourescence

Pregnant mice were dissected along the uterus and cleansed using PBS. Uterus were labeled using anti mouse Qa-2 FITC for 30 min in CO2 incubator. They were then inspected for Qa-2 signal (green) using fluorescence microscope.

Second Stage Research

Hsp-70 and VCAM-1 expressions of trophoblast cells were assessed on day 6, 12 and 18 using immunohistochemical methods in both anti-Qa 2 treatment group and control. Pregnant mice was first received anti-Qa 2.

RESULTS

The First Stage

There was no Qa-2 expressions after total 40ng anti-Qa 2 treatment. Qa-2 was expressed by green color under inverted fluorescence microscope.

In Figure 1, the whole trophoblasts looked green, which shows the presence of Qa-2. Figure 2 showed a gleam fluorescence indicating the lack of Qa-2.

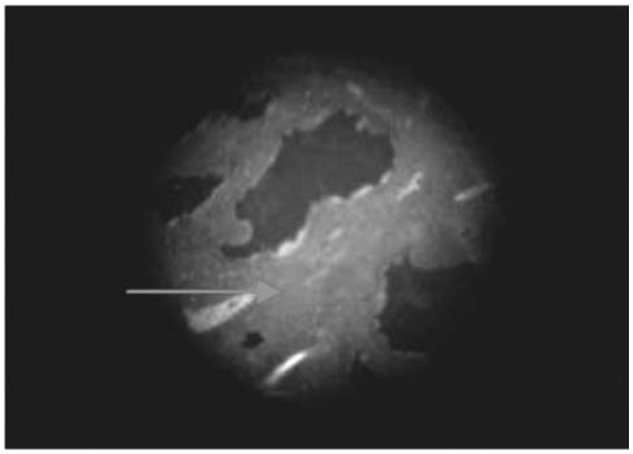

Figure 1. Positive Qa-2 as a control.

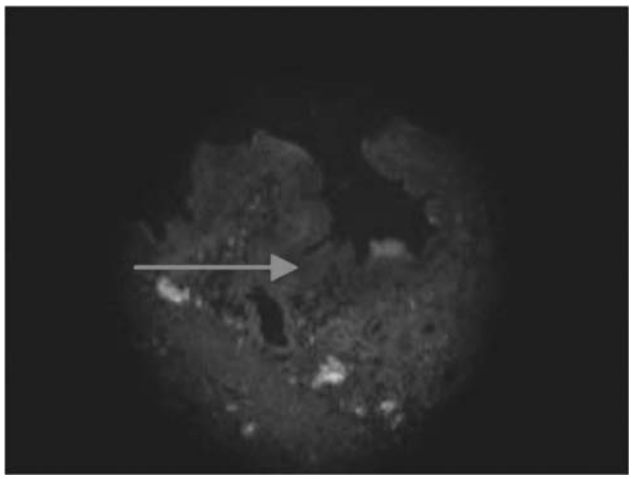

Figure 2. Negative Qa-2 as a control.

The Second Stage

Control group (positive Qa-2 expression)

This group consist of 30 pregnant mice. Ten mice were dissected each on day 6, 12 and 18 of pregnancy respectively. We assessed Hsp-70 and VCAM-1 in trophoblast cells using immunohistochemistry method.

Treatment group (negative Qa-2 expresssion)

This group consist of 30 pregnant mice received 40 ng anti-Qa-2. Ten mice were dissected each on day 6, 12 and 18 of pregnancy respectively. We assessed Hsp-70 and VCAM-1 in trophoblast cells using immunohistochemistry method.

Hsp-70 expression in treatment and control group

- Day 6 pregnant mice
  Hsp-70 expression was being assessed in treatment and control group mice using light microscope (400x). Hsp-70 was significantly more expressed in control group (31.8 ± 6.92% of field of view) compared with those of treatment group (25.00 ± 4.00% of field of view) with p=0.02 (95% confidence interval).

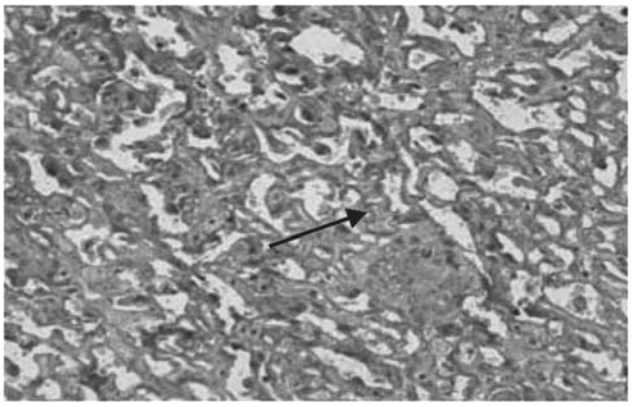

Figure 3. Hsp-70 expression in Qa-2 positive pregnant mice.

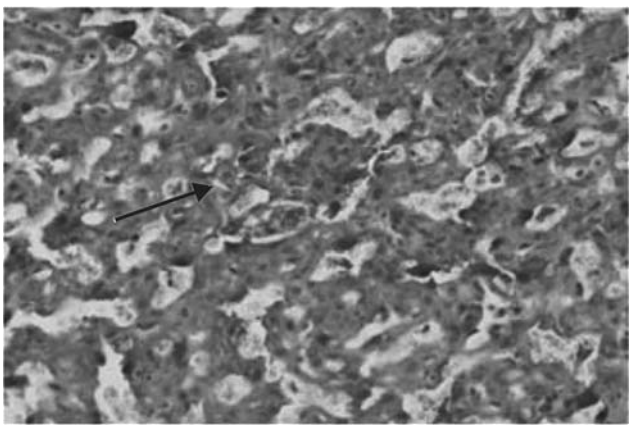

Figure 4. Hsp-70 expression in Qa-2 negative pregnant mice.

Table 1 shows average distribution of trophoblastic Hsp-70 expression.

Table 1. The distribution of Hsp-70 expression in control and treatment group on day 6.

Group	N	average distribution of Hsp-70 (% of field of view)
Treatment	10	25.00 ± 4.00
Control	10	31.8 ± 6.92

**t-test*

- Day 12 pregnant mice
  Hsp-70 was significantly more expressed in control group (2.04 ± 0.26% of field of view) compared with those of treatment group (1.48 ± 0.16% of field of view) with p≤0.01 (95% confidence interval).
- Day 18 pregnant mice
  Hsp-70 was significantly more expressed in control group (2.03 ± 0.08% of field of view) compared with those of treatment group (0.14 ± 0.01% of field of view) with p=0.049 (95% confidence interval).

VCAM-1 Expression in Treatment and Control Group

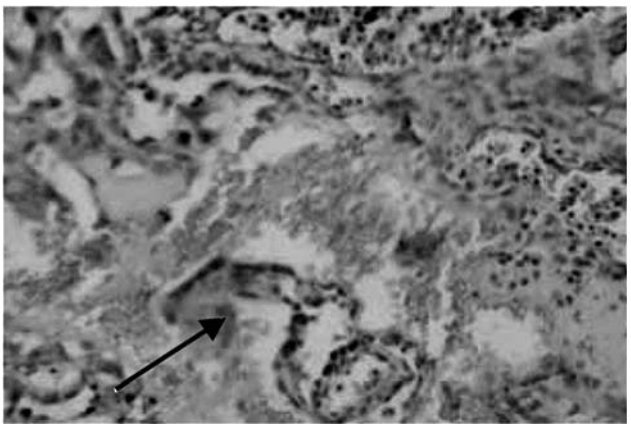

Figure 5. VCAM-1 expression in Qa-2 positive pregnant mice.

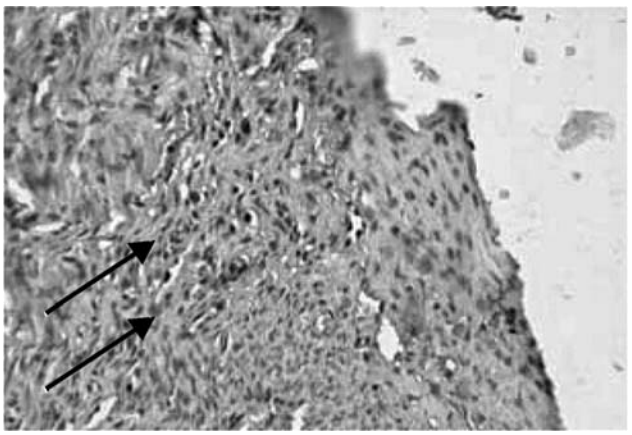

Figure 6. VCAM-1 expression in Qa-2 negative pregnant mice.

- Day 6 pregnant mice
  VCAM-1 expression was assessed in treatment and control group mice using light microscope (400x). VCAM-1 was significantly more expressed in control group (31.80 ± 6.92% of field of view) compared with those of treatment group (25.00 ± 4.00% of field of view) with p=<0.01 (95% confidence interval).

Table 2 shows average distribution of trophoblastic VCAM-1 expression.

Table 2. The average distribution of VCAM-1 expression in control and treatment group on day 6.

Group	N	average distribution of VCAM-1 expression (% of field of view)
Treatment	10	25.00 ± 4.00
Control	10	31.80 ± 6.92

- Mice on their second week pregnancy (day 12 of pregnancy)
  The average distribution of VCAM-1 expression in the negative Qa-2 mice was higher (16.60 ± 4.22% of field of view), compared to the positive Qa-2 mice (1.40 ± 0.69% of field of view).
  Analysis of VCAM-1 expression variables was performed using a normal distribution test (Kolmogorov-Smirnoff) on positive Qa-2 mice and
- Day 18 pregnant mice
  VCAM-1 was significantly more expressed in control group (97.70 ± 3.62% of field of view) compared with those of treatment group (1.40 ± 0.51% of field of view) with p=<0.01 (95% confidence interval).

DISCUSSION

This is an experimental study using Mus musculus to assess the role of class Ib MHC (HLA-G/Qa-2), Hsp-70 and VCAM in preeclampsia. Human experiments can only be performed by studying trophoblast taken after birth, while studies on first and second trimesters were hindered by ethical problems. Due to invasive method needed to obtain the trophoblast cells therefore, early pregnancy study can only be performed on animal models.

Mice Qa-2 is Functional Homologue of Human HLA-G

Both HLA- and Qa-2 are class Ib MHC molecule and have similarity in structure, non-peptide binding, membrane bound and soluble isoform, expression by pre-implanted embryo, similar role with pre-implan-ted embryo growth rate, good fetal survival, and adequate birthweight. The only difference is their linkage to membrane; Qa-2 is GPI linked, while HLA-G is not.[5]

Higher Hsp-70 Protein Expression in control group

In normal pregnancy, there is an oxidative balance throughout pregnancy. In pathologic pregnancy, such

as preeclampsia, the excessive production of reactive oxygen species (ROS) can occur on certain parts of placental development. This might decrease perfusion function and cause accumulation of placental debris due to apoptosis.

In this study, there was significant increase in trophoblast tissue Hsp-70 expression on the 1st, 2nd and 3rd week of gestation, on negative Qa-2 pregnant mice compared to its expression in positive Qa-2 pregnant mice (control group). This was in accordance with Padmini (*et al*) study. Padmini compared Hsp-70 expression (constitutive HSC-70), and inducible (Hsp-70) form) with oxidative stress condition in preeclampsia compared with those in control. HSC-70 and placental Hsp-70 concentration was significantly higher in preeclampsia ($p<0,001$) compared to control group. High concentration Hsp-70 in placenta of preeclampsia indicated the multiple protection effect to the cellular stress response.[10] Hung (*et al*) concluded that the increase of Hsp-70 in placental was a compensatory response toward ischemia, such as oxidative stress in preeclampsia.[11]

Higher VCAM-1 Protein Expression in Control Group

Endothelial dysfunction is one of preeclampsia pathogenesis and is expressed through VCAM-1, apart from expression of some other endothelial cell molecule. The increase of VCAM-1 expression in future preeclampsia patients at later gestational age, proved endothelial cell involvement weeks before clinical symptom of preeclampsia appear. This may serve as a parameter to predict preeclamptic risk in pregnancy.[12]

In this study, VCAM-1 was significantly more expressed in control group compared with those of treatment group day 6, 12 and 18 of pregnancy. The increased of VCAM-1 expression corroborates that endothelial damage occurs in early and worsen as pregnancy becomes older.

Austgulen (*et al*) reported higher VCAM-1 expression in preeclamptic woman compared with control. Zhon (*et al*) reported the human trophoblastic failure to imitate vascular adhesion phenotype in preeclampsia. The changes of VCAM-1 that happened in the first trimester may be related to its increase in the second trimester, and might leads to the development of preeclampsia in the third trimester.[13] John T (*et al*) reported higher VCAM-1 expression in placental bed (endothelium of spiral artery, as well as trophoblastic cells) in preeclampsia compared with control.[14]

CONCLUSION

Hsp-70 and VCAM-1 protein expression is higher in low, or lack of expression of class Ib MHC protein (HLA-G/Qa-2) of the trophoblast tissue might be associated with endothelial dysfunction in preeclampsia.

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