Research Report

The Influence of Low HLA-G Protein Expression on Hsp-70 and VCAM-1 Profile in Preeclampsia

Pengaruh Ekspresi Protein Rendah HLA-G pada Hsp-70 dan VCAM-1 Profil Preeklampsia

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Abstract

Objective: To analyze HLA-G, Hsp-70, and VCAM-1 proteins expression on trophoblast in women with preeclampsia and women with normal pregnancy, and to assess causality relationship between low HLA-G expression and the increased Hsp-70 and VCAM-1 expression in preeclampsia.

Method: Observational analytic study with cross sectional design.

Results: Trophoblastic HLA-G expression in pregnant women with preeclampsia is lower (p = 0.01) than its expression in women with normal pregnancy. The expressions of Hsp-70 (p = 0.02) and VCAM-1 (p = 0.00) in preeclampsia are higher, compared to normal pregnancy. Regression analysis showed that low HLA-G is a predictor for the increase of trophoblastic Hsp-70 and VCAM-1.

Conclusion: Low trophoblastic HLA-G in is a predictor for endothelial dysfunction in women with preeclampsia.

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

Abstrak

***Tujuan**: Penelitian ini bertujuan untuk menganalisis ekspresi protein HLA-G, Hsp-70 dan VCAM-1 pada trofoblas perempuan preeklampsia dan perempuan hamil normal. Menganalisis adakah hubungan kausatif adanya penurunan HLA-G terhadap peningkatan ekspresi Hsp-70 dan VCAM-1 pada preeklampsia.*

***Metode**: Jenis penelitian observasional analitik dengan rancang bangun potong lintang.*

***Hasil**: Pada trofoblas ibu hamil preeklampsia ekspresi HLA-G lebih rendah (p = 0,01) dibandingkan ekspresinya pada perempuan hamil normal. Ekspresi Hsp-70 (p = 0,02) dan VCAM-1 (p = 0,00) pada preeklampsia lebih tinggi dibandingkan dengan perempuan hamil normal. Dengan analisis regresi terbukti bahwa HLA-G yang rendah merupakan prediktor untuk terjadinya peningkatan Hsp-70 dan VCAM-1 pada trofoblas.*

***Kesimpulan**: HLA-G yang rendah pada trofoblas merupakan prediktor untuk terjadinya kerusakan endotel pada perempuan preeklampsia.*

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

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INTRODUCTION

Preeclampsia is a maternal disease marked by hypertension and proteinuria after 20 weeks of gestational age. In Indonesia, the incidence of preeclampsia is in the range of 3 - 10%, and contributes up to 39.5% in the maternal cause of death in 2001, and rose significantly to 55.56% in 2002.[1] In Dr. Moewardi hospital Surakarta, preeclampsia related maternal death toll was 25, from the total of 37 maternal deaths in 1956 labor, in 2008.[2]

Although various treatment had been sucessfully developed, preeclampsia related maternal morbidity and mortality are yet to show any significant improvement. This condition was partly caused by the lack of understanding of its ethiology and pathogenesis/ mechanism. At present, many theories have tried to explain the pathogenesis of preeclampsia, from genetic predisposition, trombophilia, endocrinopathy, vasculopathy, placental ischaemia, oxidatif stress, to immune maladaptation.[3]

In normal pregnancy, the fetus is a semialograf system (which brought with it, the father's antigen), accepted by mother. This is a phenomenon brought forward by the maternal tolerance in its immune respons mechanism, both local (between the trophoblast tissue and the immune cells from maternal decidua in early period of pregnancy) and systemic (between the sintitiotrophoblast and maternal immune cells in circulation/systemic).[4,5] The cause for this immune tolerance is not yet understood, yet there is a possibility that the placenta possesses a unique type of *human leucocyte Antigen* and specific inflammatory cell (*large granular lymphocyte*/LGL), thus, the trophoblast nei-

ther expresses the class Ia/classic *major histocomatibility complex* (MHC), namely the HLA-A and HLA-B, or the class II MHC molecule. On the other hand, the trophoblast does express class Ib/ non classic HLA-A such as HLA-G, HLA-E, HLA-F and HLA-C (class Ia). Moreover, there is an abundance of uterine *Natural Killer* (NK) cells in the decidua, which expresses a range of diverse receptors known to recognize the class Ib HLA molecule being expressed by the throphoblast, and thus helps the trophoblast to elude from an NK cell induced lysis.[6,7]

Should there be an absent, or lack there of, HLA-G expression, the ability of the trophoblast ability to invade uterus will be reduced, or hindered (as the trophoblast is assumed to be a *non self* entity). And in the same instant, the maternal NK cell will destroy the efore mentioned trophoblast. Without the proper trophoblast invasion, maternal artery would not be remodeled, and caused a reduced uteroplacental flow, which resulted in placental hypoxia/ischemia and preeclampsia. This circumstance supports the hypothesis that preeclampsia is caused by an abnormality in maternal and fetal local immune recognition at the uteroplacental level.[8]

Heat Shock Protein (Hsp) is known to protect cells from various cytotoxic disturbances. Induction of increased stress protein is related to organ exposure to stress. Induced stress proteins can protect cells from stress-triggered damage by preventing protein denaturation and, or, by repairing the damage (as a chaperone protein).

During physiologic stress, Hsp over expression plays an important role in cell protection from apoptosis. As metabolically active system are needed to preserve the pregnancy, there is a possibility that the placenta tissue, especially in preeclampsia, is suffering from a redundant stress; this could be the reason for the increase in Hsp production.

Data reported by Xu et al (1997) showed that Hsp play a role, both in the repair of proteins denaturated by oxidative stress, and in the folding of new polypeptide chain. This underlined the Hsp-70 importance in preserving homeostasis during oxidative stress, and oxydan then free radical.[9] These can trigger a defense mechanism from oxidative stress by increasing Hsp-70 expression, and in preeclampsia, protect the cell from apoptosis. It was suggested that Hsp-70 increase is related with placenta in preeclamptic women. The increased Hsp expression showed a multiple protection effect on cell response to stress.[10]

Dysfunctional endothelial cells underwent activation by producing surface cell proteins that facilitate adhesion with numerous inflammatory cells. This induction process is facilitated by pro inflammatory cytotoxin, like TNF-α and IL-1 β produced by various inflammatory cells and activated endothelial cells. A handful substance called the *leucocyte-endhotial adhesion molecule* has been identified, including *E-selectin*, VCAM-1 and ICAM-1.[11]

This study was intended to analyze HLA-G, Hsp-70 and VCAM-1 proteins expression in the trophoblast of preeclamptic, and normal pregnancy, and to assess any causative relationship between HLA-G, Hsp-70 and VCAM-1 expressions.

METHOD

This study was conducted in the Obgyn Department of Medical Faculty of Sebelas Maret University/Dr. Moewardi hospital Surakarta, from October 2008 to March 2009. Immunohistochemical staining and observation was conducted in Pathology Anatomy Department Medical Faculty of Airlangga University Surabaya.

This is an *observational analytic* study with *cross sectional* design. The study was conducted on fullterm pregnant women with severe preeclampsia, and on normal pregnant women, to analyze trophoblastic HLA-G, Hsp-70 and VCAM-1 proteins expression.

The number of sample needed to test the hypothesis was determined using replication from Steel and Torrie.[12] Based on that formula, the number of sample needed for each group was 10.

Inclusion criteria

Primigravida age ≤ 35 years of age with good nutritional status, full-term pregnant with single fetus, without chronic hypertension, did not suffer from early rupture of the membrane, without heart disease, kidney disorder, diabetes mellitus or liver disorder, infection or prolonged labor.

Exclusion criteria

Patients who did not wish to participate in the study, or those who initially participate and drop out, or died, in the duration of the study.

To obtain homogenous samples, we perform tests on subjects who underwent labor in dr. Moewardi hospital Surakarta fulfilling our inclusion criteria to determine existence of any exclusion criteria. Those who passed, were offered to enroll in our study.

Two groups of samples were obtained. The first is the case group, labor with severe preeclampsia, while the second group consisted of labor with normal pregnancy, which served as our control group. Immunohistochemical studies were performed on trophoblasts of each sample to assess their HLA-G, Hsp-70 and VCAM-1 proteins expression. The result was analyzed using t test to analyze any causative relation, while linier regression analysis was used to determine their usability as predictor.

RESULT

The characteristic of Research Subject

Table 1. Data distribution of research subject.

Variable	n	Min	Max	Average	SD
Maternal age (year)	20	17.00	34.00	25.85	4.77
Gestational age (week)	20	37.00	41.00	38.55	1.19
Systole Blood Pressure (mmHg)	20	90.00	180.00	140.00	31.11
Diastole Blood Pressure (mmHg)	20	60.00	120.00	93.50	19.26
Hemoglobin (gr/dl)	20	10.40	14.20	12.39	1.25
Blood Glucose level (mg/dl)	20	58.00	113.00	84.91	15.49
Ureum (mg/dl)	20	12.00	34.00	19.35	6.01
Creatinine (mg/dl)	20	0.60	1.30	0.78	0.18
SGOT (U/I)	20	10.00	42.00	23.40	7.71
SGPT (U/I)	20	7.00	55.00	21.45	10.29
Leucocytes count ($10^3/\mu1$)	20	4200.00	8000.00	6.14	1197.98

Table 2. Test for Mean Difference on Research Subject, Based on type of group (Preeclampsia and Normal pregnancy Group).

Variable	Group	n	Mean	SD	p score
Maternal age (years)	Preeclampsia	10	27.50	5.58	0.12
	Normal	10	24.20	3.29	
Gestational age (weeks)	Preeclampsia	10	38.30	1.41	0.36
	Normal	10	38.80	0.91	
Systole Blood Pressure (mmHg)	Preeclampsia	10	169.00	8.75	0.00*
	Normal	10	111.00	9.94	
Diastole Blood Pressure (mmHg)	Preeclampsia	10	111.00	5.67	0.00*
	Normal	10	76.00	8.43	
Hemoglobin (g/dl)	Preeclampsia	10	12.22	1.42	0.54
	Normal	10	12.57	1.10	
Glucose Blood level (mg/dl)	Preeclampsia	10	83.30	17.51	0.65
	Normal	10	86.53	13.95	
Ureum (mg/dl)	Preeclampsia	10	20.59	7.74	0.37
	Normal	10	18.12	3.59	
Creatinine	Preeclampsia	10	0.83	0.24	0.28
	Normal	10	0.74	0.06	
SGOT (U/I)	Preeclampsia	10	24.20	8.91	0.65
	Normal	10	22.60	6.68	
SGPT (U/I)	Preeclampsia	10	22.90	12.45	0.54
	Normal	10	20.00	8.00	
Leucocytes count (103/µ1)	Preeclampsia	10	5800.00	1187.90	0.21
	Normal	10	6480.00	1166.95	
Parity	Preeclampsia	10	1.00	0.00	-
	Normal	10	1.00	0.00	

**Significant value p < 0.05*

Trophoblastic HLA-G, Hsp-70 and VCAM-1 Expressions in Preeclampsia and Normal Pregnancy

HLA-G expressions in Preeclampsia were lower, compared to normal pregnancy (p = 0.01).

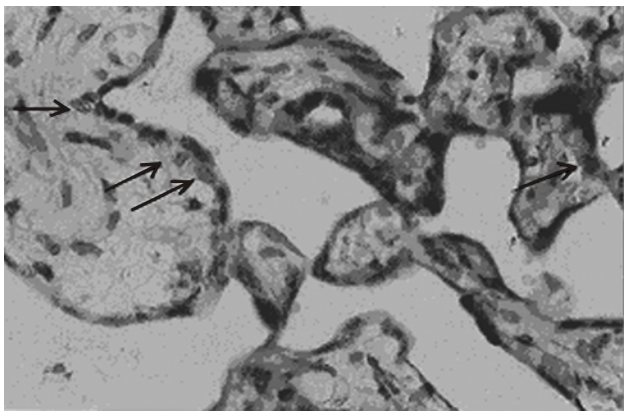

Figure 1. HLA-G in preeclampsia.

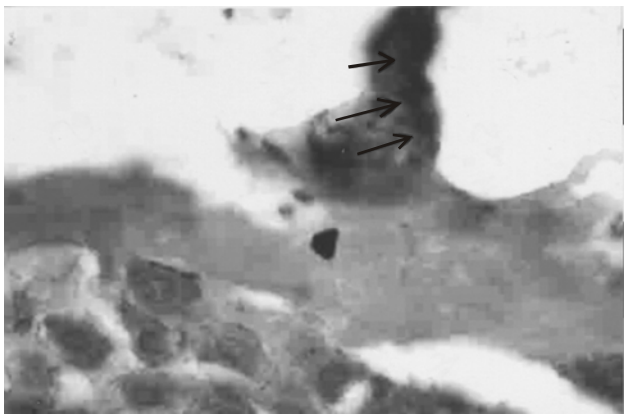

Figure 2. HLA-G in normal pregnancy.

Hsp-70 expressions in preeclampsia were higher, compared to normal pregnancy (p = 0.02).

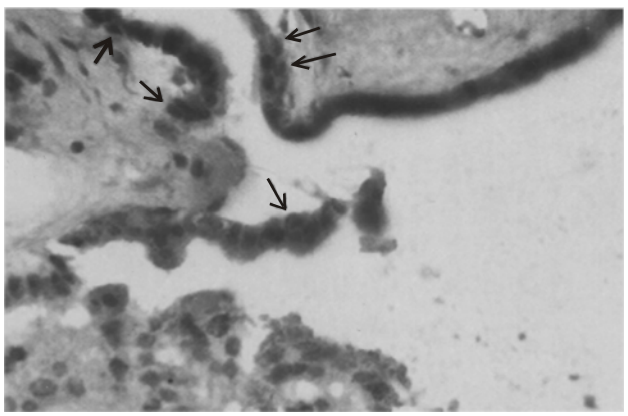

Figure 3. Hsp-70 in preeclampsia.

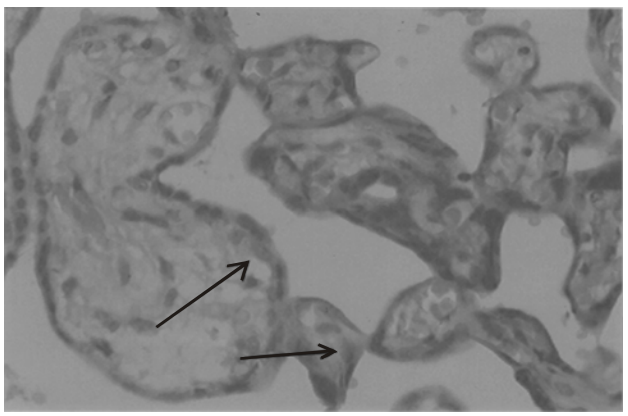

Figure 4. Hsp-70 in normal pregnancy.

Trophoblastic VCAM- 1 expressions in preeclampsia were highercompared to normal pregnancy (p = 0.00).

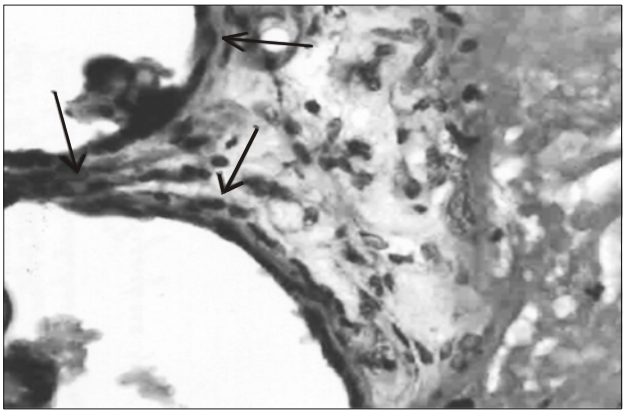

Figure 5. VCAM-1 in preeclampsia.

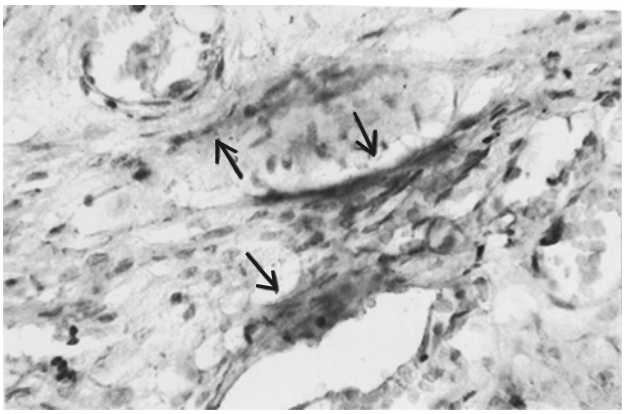

Figure 6. VCAM-1 in normal pregnancy.

Linier Regression Analysis

- Linear regression analysis for HLA-G and Hsp-70 expression resulted in p = 0.01. Thus the HLA-G expression is a predictor for the increased Hsp-70 expression in Preeclampsia etiopathogenesis.
  In Figure 7 we can see that the decrease in HLA-G expression resulted in a linear increase in Hsp-70 expression.

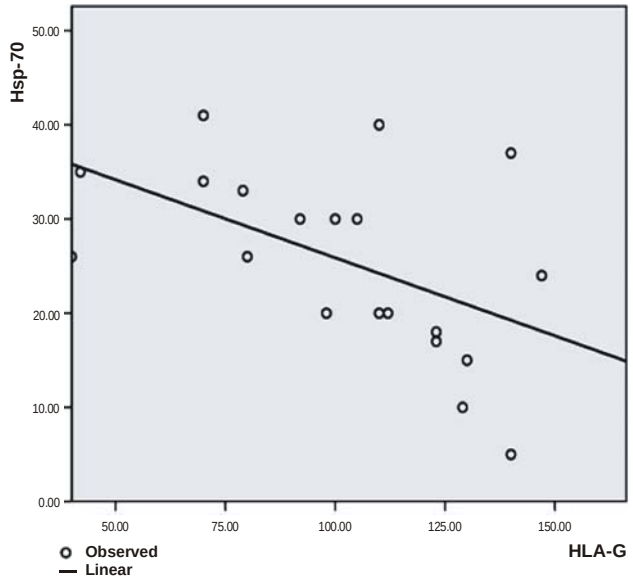

Figure 7. Graphic of the relation between HLA-G and Hsp-70 in Preeclampsia etiopathogenesis.
X = predictor = HLA-G
Y = dependent variable = Hsp-70

- Linear regression analysis for Hsp-70 and VCAM-1 expression resulted in a p value of 0.04. Thus the Hsp-70 expression is a predictor for the increased VCAM-1 expression in Preeclampsia etiopathogenesis.
  In Figure 8 we can see that the increase in Hsp-70 expression resulted in a linear increase in VCAM-1 expression.

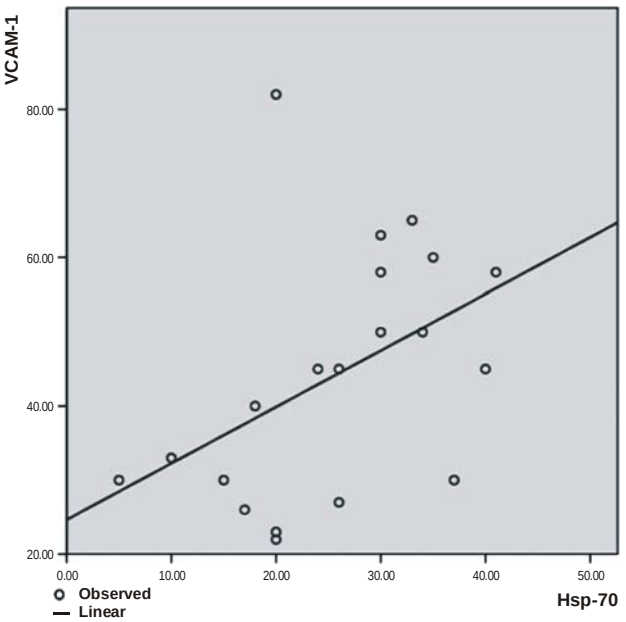

Figure 8. Graphic of the relation between Hsp-70 and VCAM-1 expression in Preeclampsia etiopathogenesis.
X = predictor = Hsp-70
Y = dependent variable = VCAM-1

DISCUSSION

Low trophoblastic HLA-G Protein Expression is a factor for preeclampsia etiopathogenesis

Trophoblast is the only conception cell in direct contact with maternal tissue or blood. It is genetically identical with the fetus tissue. In normal pregnancy, this tissue expresses a large amount of HLA-G, a non classic class Ib MHC molecule, during its invasion into the uterus. Since HLA-G is numeric, it is considered as *self*, and therefore, if sufficiently expressed, does not trigger maternal immunologic response. The lack, or absent, of HLA-G expression prevents trophoblastic invasion into maternal tissue, and proper vascular invasion. This failure creates a placental defect resulted in a decreased uteroplacental blood flow that can be seen on preeclampsia.[13]

Result of our study showed that trophoblast taken from preeclamptic patients had lower HLA-G expression mean of distribution (82.00 ± 26.45%/field of view) compared to normal pregnancy (122.00 ± 20.74 %/field of view). Statistic analysis also confirmed a significant difference with p = 0.01 (< 0.05). This result confirmed Goldman's observation (2000) that 9 from 10 pregnant mothers with preeclampsia showed low HLA-G expression. Therefore, trophoblast lacking HLA-G expression is vulnerable to maternal immune system attack. This defective trophoblast could not effectively invades maternal's spiral artery, and thus the later developed vascular system could not provide sufficient nutrition needed by the developing placenta. This inadequately perfused placenta may initiate a series of systemic events known as preeclampsia.[8]

Yie (2004) conducted a study on serum and placental HLA-G concentration. The result showed that both serum and placental HLA-G on women with preeclampsia was significantly lower (median = 0.026 ng/ml in serum and median = 0.026 ng/ml in placenta) compared to the concentration found on women with normal pregnancy (median = 0.093 ng/ml in serum and median 0.088 ng/ml in placenta). The conclusion of this study was that the decrease of HLA-G expression in human placenta and (subsequently) the decrease of HLA-G released in maternal circulation may impair the feto-maternal immune relationship. This may resulted in preeclampsia. It was concluded that the immune mechanism might play a central role in first stage of preeclampsia pathogenesis. The limited and naturally monomorphic HLA-G expression had led many to hypothesized that HLA-G could be formulated as a universally accepted identity card, allowing the trophoblast to avoid rejection.

The lack of, or significantly low, HLA-G expression on early stages of pregnancy may result in total fetal loss. Pregnancy with a mild degree of low HLA-G expression can continue into the first trimester with placental ischemia effect, which may later develop into preeclampsia.[14]

Low, or altered, HLA-G expression may result in functional disorder. Low placental HLA-G concentration in the first trimester of pregnancy in mixed lymphocytes reaction can multiply the amount of both type Th-1 cytokine and allocytotoxic T lymphocyte (CTL) released. While on the other hand, a high/sufficient concentration of HLA-G can increase the type Th-2 cytokine, and decrease the type Th-1 cytokine and CTL response. The effect of low HLA-G can influence placentation, resulted in weak intertitial trophoblast invasion and scant endovascular invasion (meanwhile the endovascular trophoblast cell itself expressed HLA-G protein to protect cells from NK cell related lyses) and also systemically influenced endothelial cells in pregnant women who would later emerge as preeclampsia.[15]

The TNF-α, IL-2 and IFN-γ producing type Th-1 T cell becomes the tempest of pregnancy, while the type Th-2 T cell that secreted IL-3, IL-4, IL-10, IL-13 and TNF-β2 plays a protecting role. Study by Yie (2004) involving a reaction test by mixing lymphocyte in vitro, found that high concentration of HLA-G produced a type Th-2 response, while the opposite produced a type Th-1 response. Low HLA-G might disturb its abi-lity to protect feto-placental unit from generation of T cell cytotoxic and, or, lyses by NK cell.[14]

Unlike Yie (2004), immunohistochemical study by Datema et al (2003) stated that there was no difference between HLA-G expression in preeclamptic and normal placenta. This opposite observation may come from the small number of sample used in Datea study, which consisted of 4 placentas each, for the preeclamptic, and normal group.[16]

High trophoblastic HSP-70 protein expression is the etiopathogenesis of preeclampsia

Hsp-70 is known to be able to protect cell from cytotoxic disturbance and is expressed as a response to stress, acted as a chaperone for cellular protein, and then binds the emerging polypeptide to prevent premature folding, and to translocate the protein into the organels.

Over expression of Hsp-70 has an important meaning on cell protection during physiologic stress in apoptosis process. Just as metabolically active tissue is important in maintaining pregnancy, so is the placenta system in preeclampsia suffering from tremendous stress. This is also the reason that triggers the Hsp-70 production. The result is the increased Hsp-70 and alterations in placental endothelial cells. The increased of placental Hsp-70 expression in preeclampsia might demonstrate a multiple protection effect on cellular response toward stress.

In this study, we found that the trophoblastic Hsp-70 expression of pregnant women with preeclampsia (31.90% ± 6.24%/field of view) is higher, compared to normal pregnancy(1920 ± 8.80%/scope of view), a difference with statistic significance, with p = 0.02 (p < 0.05). This result was similar to those obtained earlier, such as Padmini (2008) which studied the Hsp-70 expression either in constitutive (HSC-70) or inducible (Hsp-70) form, in relation to oxidative stress condition on placenta tissue, in preeclamptic, and normotensive pregnancy as a control group. The result was that the placental concentration of HSC-70 and Hsp-70 was significantly higher in preeclampsia (p < 0.001) compared to the control group. It was deducted that high level of Hsp-70 is related with placenta of preeclamptic women. The increased expression showed a multiple protection effect of celllular response to stress.[10] Hung and colleagues (2001) concluded in their study that the increase of Hsp-70 in placental tissue was a compensatory response to ischemia as an example of oxidative stress in preeclampsia.[17]

That condition (the increase of Hsp-70) in trophoblast might indicate the multiple protection effect of cellular response to the stress.

Higher expression of VCAM-1 protein in the trophoblast tissue is an etiopathogenesis factor of preeclampsia

The increase of serum and placental VCAM-1 concentration in women during the second trimester of pregnancy, who later developed preeclampsia, was indicative of an endothelial alteration in women with

preeclamptic tendency, weeks before the onset of clinical symptoms. This finding gave a rationale that there was an increased endothelial damage, and hopefully could serve as a predictive parameter in identifying pregnant women with the risk for preeclampsia.[18]

Our study found higher trophoblastic VCAM-1 protein expression in preeclamptic pregnancy compared to the control group.

Austgulen et al reported an increased VCAM-1 expression in pregnant women with preeclampsia. Zhon and colleagues stated that preeclampsia is related to a failure of human trophoblast to imitate vascular phenotype adhesion. The alteration of VCAM-1 during the first trimester of pregnancy might be mechanically related with the increase of its expression in the second trimester, and ended in the development of preeclampsia during the third trimester of pregnancy.[18]

John T and colleagues (2003) compared VCAM-1 expression in *placental bed* (endothelium of the spiral artery, as well as the trophoblast cells) from 20 pregnant women with preeclampsia and 20 women with normal pregnancy using Immunohistochemical technique. VCAM-1 expression in pregnant women with preeclampsia was higher than normal pregnancy; although the difference was not statistically significant.[19]

CONCLUSION

In preeclampsia, the HLA-G protein expression of is lower than that of normal pregnancy. Hsp-70 and VCAM-1 protein expression in preeclampsia are higher compared to that of normal pregnancy, and there is a causative relationship between the low expression of HLA-G, to the increase of Hsp-70 and VCAM-1 protein expression in women with preeclampsia.

SUGGESTION

In order to obtain a more optimum result related to the etiopathogenesis of preeclampsia, the writer suggested a series of examination to exclude factors that can result in an altered HLA-G protein expression (increased, lowered, or a lack there of) in early pregnancy period, such as examination for HLA-G gene polymorphism, virus infections (i.e. *Human Citomegalovarious* (HCMV), herpes virus simplex 1), and examination for specific immunity on multiparous women who changed her partner, or was pregnant by a different partner.

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