Circulating anti-heat-shock-protein antibodies in normal pregnancy and preeclampsia

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Received: 27 November 2008 / Revised: 17 January 2009 / Accepted: 21 January 2009 / Published online: 11 February 2009
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Abstract It has been previously reported that circulating anti-heat-shock-protein (Hsp) antibody levels are elevated in cardiovascular disorders. The aim of the present study was to determine circulating antihuman Hsp60, antimycobacterial Hsp65, and antihuman Hsp70 antibody levels in healthy pregnant women and preeclamptic patients and to investigate their relationship to the clinical characteristics of the study subjects, as well as to the markers of inflamma-

tion (C-reactive protein (CRP)), endothelial activation (von Willebrand factor antigen), or endothelial injury (fibronectin), oxidative stress (malondialdehyde) and to serum Hsp70 levels. Ninety-three preeclamptic patients and 127 normotensive healthy pregnant women were involved in this case control study. Serum anti-Hsp60, anti-Hsp65, anti-Hsp70, and Hsp70 levels were measured with enzyme-linked immunosorbent assay (ELISA). Serum CRP levels were determined by an autoanalyzer using the manufacturer's kit. Plasma von Willebrand factor antigen levels were quantified by ELISA, while plasma fibronectin concentration by nephelometry. Plasma malondialdehyde levels were measured by the thiobarbituric-acid-based colorimetric assay. For statistical analyses, nonparametric methods were applied. Anti-Hsp60, anti-Hsp65, and anti-Hsp70 antibodies were detected in all of our serum samples. There were no significant differences in serum anti-Hsp60, anti-Hsp65, and anti-Hsp70 antibody levels between the control and preeclamptic groups. Serum levels of Hsp70 and CRP, as well as plasma levels of VWF antigen, fibronectin, and malondialdehyde, were significantly higher in preeclamptic patients than in normotensive healthy pregnant women. Serum anti-Hsp60 antibody levels showed significant correlations with serum anti-Hsp65 antibody levels both in the control and the preeclamptic groups (Spearman $R=0.55$ and 0.59 ; $p<0.001$, respectively). However, no other relationship was found between clinical features (maternal age, smoking status, parity, body mass index, gestational age at blood draw, systolic and diastolic blood pressure, gestational age at delivery, and fetal birth weight) and measured laboratory parameters of the study subjects and serum anti-Hsp antibody levels in either study group. In conclusion, anti-Hsp60 and anti-Hsp70 antibodies as naturally occurring autoantibodies are present in the peripheral circulation of

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healthy pregnant women. Nevertheless, humoral immunity against heat shock proteins was not associated with preeclampsia. Further studies are warranted to explore the role of heat shock proteins and immune reactivity to them in the immunobiology of normal pregnancy and preeclampsia.

Keywords Antibody · Anti-Hsp · Heat shock protein · Preeclampsia · Pregnancy

List of abbreviations

ANCOVA	analysis of covariance
AU	arbitrary unit
BMI	body mass index
CRP	C-reactive protein
EDTA	ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay
HELLP	hemolysis, elevated liver enzymes, and low platelet count
HRP	horseradish peroxidase
Hsp	heat shock protein
Ig	immunoglobulin
IL	interleukin
OPD	<i>o</i> -phenylene-diamine
PBS	phosphate-buffered saline
T _h	T helper
VWF:Ag	von Willebrand factor antigen

Introduction

Preeclampsia is a severe complication of human pregnancy with a worldwide incidence of 2–10% (Duckitt and Harrington 2005). It is one of the leading causes of maternal as well as perinatal morbidity and mortality, even in developed countries. Despite intensive research efforts, the etiology and pathogenesis of preeclampsia are not completely understood. Increasing evidence suggests that an excessive maternal systemic inflammatory response to pregnancy with systemic oxidative stress and resultant endothelial damage plays a crucial role in the pathogenesis of the disease (Redman et al. 1999). The development of preeclampsia is influenced by both genetic and environmental risk factors, suggesting its multifactorial inheritance (Roberts and Gammill 2005a).

Heat shock proteins (Hsps) are ubiquitous and phylogenetically conserved molecules. They are usually considered to be intracellular proteins with molecular chaperone and cytoprotective functions (Hightower 1991). However, as immunodominant molecules, heat shock proteins may be the targets of the immune response. Antihuman Hsp60, antimycobacterial Hsp65, and antihuman Hsp70 antibodies have been detected in the peripheral circulation of healthy

nonpregnant individuals (Pockley et al. 1998, 1999). Serum anti-Hsp65 antibody levels were raised in subjects with borderline hypertension, while anti-Hsp65 and anti-Hsp70 antibody levels were both elevated in established hypertension (Frostegard et al. 1997; Pockley et al. 2000, 2002). Increased concentrations of circulating antibodies against human Hsp60 and mycobacterial Hsp65 have been reported to be associated with the presence, severity, and progression of atherosclerosis-related cardiovascular diseases (Xu et al. 1993, 1999; Birnie et al. 1994, 1998, 2005; Hoppichler et al. 1996; Burian et al. 2001; Prohaszka et al. 2001; Zhu et al. 2001; Veres et al. 2002a). In addition, antibodies against Hsp60/65 can mediate endothelial cytotoxicity (Schett et al. 1995; Mayr et al. 1999). Elevated circulating anti-Hsp70 antibody levels have also been observed in atherosclerotic cardiovascular disorders (Chan et al. 1999; Gromadzka et al. 2001; Yuan et al. 2005), but the results are still controversial (Kocsis et al. 2002). Interestingly, cardiovascular diseases share many risk factors (obesity, dyslipidemia, insulin resistance) and pathogenetic features (inflammation, oxidative stress, and endothelial injury) with preeclampsia. Furthermore, women who develop preeclampsia are at increased risk of cardiovascular disorders later in life (Roberts and Gammill 2005b).

We have recently reported that serum Hsp70 levels are increased and reflect systemic inflammation, oxidative stress, and hepatocellular injury in preeclampsia (Molvarec et al. 2006, 2008). Moreover, we demonstrated that serum Hsp70 levels are significantly higher in patients with the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) than in severely preeclamptic patients without HELLP syndrome (Molvarec et al. 2007a). According to our findings, elevated serum Hsp70 level indicates tissue damage (hemolysis and hepatocellular injury) and disease severity in HELLP syndrome (Madach et al. 2008).

In the present study, we measured circulating antihuman Hsp60, antimycobacterial Hsp65, and antihuman Hsp70 antibody levels in healthy pregnant women and preeclamptic patients and investigated their relationship to the clinical characteristics of the study subjects, as well as to the markers of inflammation (C-reactive protein (CRP)), endothelial activation (von Willebrand factor antigen), endothelial injury (fibronectin), or oxidative stress (malondialdehyde) and to serum Hsp70 levels. Given that humoral immunity against heat shock proteins has been implicated in the development of cardiovascular disorders and that cardiovascular diseases share many common pathophysiological mechanisms with preeclampsia, our null hypothesis was that circulating anti-heat-shock-protein antibody levels are increased in preeclampsia and that these antibodies play a role in endothelial injury and, thus, in the pathogenesis of the disease.

Materials and methods

Study participants

Our study was designed employing a case control approach. Ninety-three preeclamptic patients and 127 normotensive (blood pressure <140 mmHg systolic and <90 mmHg diastolic) healthy pregnant women with uncomplicated pregnancies were involved in the study. This sample size provided sufficient statistical power (>80% at a type I error rate of 0.05) to detect differences in serum anti-heat-shock-protein antibody levels between cases and controls which have been observed previously in coronary heart disease (Prohaszka et al. 2001; Kocsis et al. 2002). The study participants were enrolled in the First Department of Obstetrics and Gynecology and in the Department of Obstetrics and Gynecology of Kútvolgyi Clinical Center, at the Semmelweis University, Budapest, Hungary. All women were Caucasian and resided in the same geographic area in Hungary. Exclusion criteria were multifetal gestation, chronic hypertension, diabetes mellitus, autoimmune disease, angiopathy, renal disorder, maternal or fetal infection, and fetal congenital anomaly. The women were fasting; none were in active labor, and none had rupture of membranes. The subject group is identical with that of our previously published study (Molvarec et al. 2006). However, in that study, only serum Hsp70 levels were measured and reported.

Preeclampsia was defined by increased blood pressure (≥ 140 mmHg systolic or ≥ 90 mmHg diastolic on ≥ 2 occasions at least 6 h apart) that occurred after 20 weeks of gestation in a woman with previously normal blood pressure, accompanied by proteinuria (≥ 0.3 g/24 h). Blood pressure returned to normal by 12 weeks postpartum in each preeclamptic study patient. Preeclampsia was regarded as severe if any of the following criteria was present: blood pressure ≥ 160 mmHg systolic or ≥ 110 mmHg diastolic or proteinuria ≥ 5 g/24 h. Pregnant women with HELLP syndrome were not enrolled in this study. Early onset of preeclampsia was defined as onset of the disease before 34 weeks of gestation. Fetal growth restriction was diagnosed if the fetal birth weight was below the tenth percentile for gestational age and gender, based on Hungarian birth weight percentiles (Joubert 2000).

The study protocol was approved by the Regional Institutional Committee of Medical Ethics at the Semmelweis University, and written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

Biological samples

Maternal blood samples were obtained from an antecubital vein into native as well as EDTA- or sodium-citrate-

anticoagulated tubes and centrifuged at room temperature with a relative centrifugal force of $3,000\times g$ for 10 min. The aliquots of serum and plasma were stored at -80°C until the analyses were performed.

Laboratory methods

Anti-Hsp60 and anti-Hsp65 immunoglobulin G (IgG) levels were measured by enzyme-linked immunosorbent assay (ELISA), as described previously (Prohaszka et al. 1999, 2001). In brief, plates were coated with $0.1\text{ }\mu\text{g}$ per well human Hsp60 (recombinant human Hsp60, StressGen, SPP-740) or *Mycobacterium bovis* Hsp65 (recombinant *M. bovis* Hsp65, Braunschweig, Germany). After washing and blocking (phosphate-buffered saline (PBS), 0.5% gelatine), the wells were incubated with $100\text{ }\mu\text{l}$ of serum samples diluted 1:500 (PBS, 0.5% gelatine, 0.05% Tween 20). Bound anti-Hsp60/65 antibodies were detected by antihuman IgG peroxidase-labeled antibodies (Sigma, St. Louis, MO, USA) and *o*-phenylene-diamine (Sigma).

Anti-Hsp70 (Hsp72) antibody levels were measured by ELISA. Microtiter plates were coated with $0.1\text{ }\mu\text{g}$ per well recombinant human Hsp70 (StressGen SPP-755) diluted in bicarbonate buffer (pH 9.4) and incubated at 4°C overnight. Plates were washed and nonspecific binding sites were blocked with 0.5% gelatine PBS solution for 1 h at room temperature. After washing, the wells were incubated with $100\text{ }\mu\text{l}$ of serum samples diluted in 1:100 in PBS containing 0.5% gelatine and 0.05% Tween 20 for 1 h. Binding of anti-Hsp antibodies was determined using $100\text{ }\mu\text{l}$ (1:6,000) γ -chain-specific rabbit antihuman IgG peroxidase-labeled antibodies (Sigma) for 1 h. After washing, the plates were added with goat antirabbit IgG antibody as conjugate (Sigma) for 1 h. Plates were washed; *o*-phenylene-diamine (Sigma) was added and optical density was measured at 490 nm (reference at 620 nm) and the means of duplicate wells were calculated. A serial dilution of control human serum containing high levels of anti-Hsp70 antibodies was used as standard. After correction for background absorbance, data obtained as optical density values were calculated to arbitrary unit per milliliter values related to this standard (Kocsis et al. 2002).

Serum Hsp70 levels were measured by using the ELISA Kit of R&D Systems (DYC1663E, Minneapolis, MN, USA), as we described previously (Molvarec et al. 2007b). Serum CRP levels were determined by an autoanalyzer (Cobas Integra 800, Roche, Mannheim, Germany) using the manufacturer's kit. Plasma von Willebrand factor antigen (VWF:Ag) levels were quantified by ELISA (Dakopatts, Glostrup, Denmark), while plasma fibronectin concentration by nephelometry (Dade Behring, Marburg, Germany), according to the manufacturer's instructions. Plasma malondialdehyde levels were measured

by the thiobarbituric-acid-based colorimetric assay (Placer et al. 1966).

Statistical analysis

The normality of continuous variables was assessed using the Shapiro–Wilk's W test. As the continuous variables were not normally distributed, nonparametric statistical methods were used. To compare continuous variables between two groups, the Mann–Whitney U test was applied. The Fisher exact and Pearson χ^2 tests were carried out to compare categorical variables between groups. As serum levels of anti-Hsp60, anti-Hsp65, anti-Hsp70, Hsp70, and C-reactive protein as well as plasma levels of VWF:Ag, fibronectin, and malondialdehyde showed skewed distributions, we performed analyses of covariance (ANCOVA) with logarithmically transformed data. The Spearman rank order correlation was applied to calculate correlation coefficients.

Statistical analyses were carried out using the following software: STATISTICA (version 8.0; StatSoft, Inc., Tulsa, OK, USA) and Statistical Package for the Social Sciences (version 15.0 for Windows; SPSS, Inc., Chicago, IL, USA). For all statistical analyses, $p < 0.05$ was considered statistically significant.

In the article, data are reported as median (interquartile range) for continuous variables and as number (percent) for categorical variables.

Results

Patient characteristics

The clinical characteristics of the study participants are described in Table 1. There were no statistically significant differences in maternal age and the percentage of smokers and primiparas between the two study groups. The body mass index (BMI) and gestational age at blood draw were significantly higher in the preeclamptic group than in the control group. The systolic and diastolic blood pressures were significantly higher, whereas the gestational age at delivery and the fetal birth weight were significantly lower in the preeclamptic group compared with the control group. Fetal growth restriction was absent in control subjects, whereas the frequency of this condition was 22.6% in the preeclamptic group.

Laboratory parameters

As shown in Table 1, serum levels of Hsp70 and C-reactive protein as well as plasma levels of VWF antigen, fibronectin, and malondialdehyde were significantly higher in preeclamptic patients than in normotensive healthy pregnant women. The differences in these variables between the two study groups remained significant even after adjustment for maternal age, BMI, and gestational age at blood draw in ANCOVA.

Table 1 Clinical characteristics and laboratory parameters of normotensive, healthy pregnant women and preeclamptic patients

	Controls ($n=127$)	Preeclampsia ($n=93$)	Statistical significance (p value)
Age (years)	28 (25–31)	28 (25–32)	NS
BMI at blood draw (kg/m^2)	26.0 (23.7–28.0)	29.4 (26.3–32.0)	<0.001
Smokers	2 (1.6%)	5 (5.4%)	NS
Primiparas	77 (60.6%)	59 (63.4%)	NS
Systolic blood pressure (mmHg)	110 (105–120)	170 (160–180)	<0.001
Diastolic blood pressure (mmHg)	70 (60–80)	104 (100–115)	<0.001
Gestational age at blood draw (weeks)	35 (31–37)	37 (35–39)	<0.05
Gestational age at delivery (weeks)	40 (39–40)	38 (35–39)	<0.001
Fetal birth weight (grams)	3,300 (3,100–3,800)	2,900 (1,980–3,450)	<0.001
Fetal growth restriction	0 (0%)	21 (22.6%)	<0.001
Serum CRP level (mg/l)	3.38 (1.69–7.27)	6.71 (2.76–12.69)	<0.001
Plasma VWF:Ag level (%)	129.3 (105.1–182.8) ^a	187.1 (145.6–243.1) ^b	<0.001
Plasma fibronectin level (g/l)	0.33 (0.27–0.40) ^a	0.58 (0.41–0.79) ^b	<0.001
Plasma malondialdehyde level (nmol/ml)	14.74 (9.20–18.98) ^a	18.62 (15.84–20.99) ^b	<0.001
Serum Hsp70 level (ng/ml)	0.31 (0.27–0.39)	0.55 (0.42–0.80)	<0.001
Serum anti-Hsp60 level (AU/ml)	34.7 (20.4–55.6)	33.6 (20.9–52.2)	NS
Serum anti-Hsp65 level (AU/ml)	9.6 (5.9–15.8)	8.6 (5.4–13.7)	NS
Serum anti-Hsp70 level (AU/ml)	229 (149–391)	206 (163–294)	NS

Data are presented as median (25–75 percentile) for continuous variables and as number (percent) for categorical variables

NS not significant, BMI body mass index, CRP C-reactive protein, VWF:Ag von Willebrand factor antigen, Hsp heat shock protein, AU arbitrary unit

^a $n=70$

^b $n=67$

Anti-Hsp60, anti-Hsp65, and anti-Hsp70 antibodies were detected in all of our serum samples. There were no significant differences in serum anti-Hsp60, anti-Hsp65, and anti-Hsp70 antibody levels between the control and preeclamptic groups (Table 1), even after adjustment for maternal age, BMI, and gestational age at blood draw in ANCOVA. In the group of preeclamptic patients, no statistically significant differences were observed in serum anti-Hsp60, anti-Hsp65, and anti-Hsp70 antibody levels between patients with mild and severe preeclampsia, between patients with late and early onset of the disease, or between preeclamptic patients with and without fetal growth restriction (Table 2).

Relationship of clinical characteristics and laboratory parameters of the study participants to serum anti-Hsp antibody levels

We investigated whether clinical characteristics and laboratory parameters of the study participants are related to serum anti-Hsp antibody levels by calculating the Spearman rank order correlation coefficients (continuous variables; Tables 3 and 4) or by the Mann–Whitney *U* test (categorical variables). Serum anti-Hsp60 antibody levels showed significant correlations with serum anti-Hsp65 antibody levels both in the control and the preeclamptic groups (Spearman $R=0.55$ and 0.59 ; $p<0.001$, respectively). However, no other relationship was found between clinical features (maternal age, smoking status, parity, BMI, and gestational age at blood draw, systolic and diastolic blood pressure, gestational age at delivery, and fetal birth weight) and measured laboratory parameters (serum Hsp70 and CRP levels, plasma levels of VWF antigen, fibronectin, and malondialdehyde) of the study subjects and serum anti-Hsp antibody levels either in normotensive healthy pregnant women or in preeclamptic patients.

Discussion

In this study, we reported the presence of anti-Hsp60, anti-Hsp65, and anti-Hsp70 antibodies in the peripheral circulation

of healthy pregnant women. However, neither were serum levels of anti-heat-shock-protein antibodies increased nor were these antibodies related to systemic inflammation, oxidative stress, and endothelial activation/injury in preeclampsia.

Our findings that anti-Hsp60 and anti-Hsp70 antibodies were present in all of our serum samples are in agreement with the role of these antibodies as naturally occurring autoantibodies. Such antibodies are important for initial defense against invading pathogens (Lutz and Miescher 2008). Indeed, anti-Hsp70 antibody was detected in mid-trimester amniotic fluid and its level correlated with intra-amniotic concentrations of antimicrobial immune mediators (Gelber et al. 2007). The lack of correlation between serum Hsp70 and anti-Hsp70 antibody levels is consistent with earlier observations in nonpregnant women (Pockley et al. 1998; Rea et al. 2001). The strong positive correlation between anti-Hsp60 and anti-Hsp65 levels found in our study groups might reflect the presence of cross-reactive epitopes on the target molecules.

Given the ubiquitous nature and the high degree of sequence homology between microbial and mammalian forms of heat shock proteins, these molecules could act as harmful autoantigens and may provide a link between infection and autoimmunity through molecular mimicry (Lamb et al. 1989). Most of the known risk factors of atherosclerosis (e.g., infection, hemodynamic stress (hypertension), oxidative stress) are known to induce heat shock protein expression in and/or release from the vessel wall. Cross-reactive anti-heat-shock-protein antibodies and T cells can damage vascular tissues overexpressing heat shock proteins, contributing to the development of atherosclerosis (Mandal et al. 2004). Additionally, immune sensitization to human Hsp60, possibly developed as a consequence of infection, may adversely affect pregnancy outcome (Witkin et al. 1994, 1996). Furthermore, the presence of anti-Hsp60 and anti-Hsp70 antibodies in the serum and formation of Hsp60- and Hsp70-immune complexes in the placenta were associated with preterm birth (Ziegert et al. 1999). Recently, serum anti-Hsp70

Table 2 Serum anti-Hsp antibody levels in the subgroups of preeclamptic patients

	Serum anti-Hsp60 level (AU/ml)	Serum anti-Hsp65 level (AU/ml)	Serum anti-Hsp70 level (AU/ml)
Mild preeclampsia ($n=47$)	28.9 (20.9–56.5)	8.4 (5.2–14.6)	197 (167–262)
Severe preeclampsia ($n=46$)	36.4 (19.3–47.4)	9.0 (5.6–13.3)	220 (163–338)
Late onset preeclampsia ($n=74$)	31.0 (20.9–51.0)	8.1 (5.2–13.0)	198 (155–276)
Early-onset preeclampsia ($n=19$)	38.2 (17.1–56.3)	11.6 (6.4–23.8)	263 (164–356)
Preeclampsia without IUGR ($n=72$)	35.1 (21.0–55.7)	8.9 (5.7–13.3)	206 (167–284)
Preeclampsia with IUGR ($n=21$)	26.1 (18.1–43.3)	8.2 (5.1–17.1)	213 (158–348)

Data are presented as median (25–75 percentile)

Hsp heat shock protein, AU arbitrary unit, IUGR intrauterine growth restriction

Table 3 Correlation coefficients between clinical characteristics and laboratory parameters of normotensive healthy pregnant women and serum anti-Hsp antibody levels

	Serum anti-Hsp60 level	Serum anti-Hsp65 level	Serum anti-Hsp70 level
Age	-0.05	-0.12	0.11
BMI at blood draw	-0.15	-0.08	-0.15
Systolic blood pressure	-0.04	0.02	0.04
Diastolic blood pressure	-0.01	-0.04	-0.05
Gestational age at blood draw	0.02	0.13	-0.10
Gestational age at delivery	0.04	0.06	0.10
Fetal birth weight	-0.01	-0.07	0.12
Serum CRP level	0.10	0.09	0.01
Plasma VWF:Ag level ^a	0.19	0.20	0.06
Plasma fibronectin level ^a	-0.01	0.08	-0.03
Plasma malondialdehyde level ^a	0.01	-0.08	-0.09
Serum Hsp70 level	-0.04	-0.09	-0.11
Serum anti-Hsp60 level		0.55*	0.10
Serum anti-Hsp65 level	0.55*		0.05
Serum anti-Hsp70 level	0.10	0.05	

Significant correlations are in bold

Hsp heat shock protein, BMI body mass index, CRP C-reactive protein, VWF:Ag von Willebrand factor antigen

* $p < 0.001$

^a $n = 70$

levels were found to be significantly elevated at 16 weeks of gestation in women who later gave birth to babies with birth defects, suggesting a prior increase in Hsp70 expression (Child et al. 2006).

Infection/inflammation and oxidative and hemodynamic stress have also been implicated in the pathogenesis of preeclampsia (von Dadelszen et al. 2003). The reason for the lack of association between circulating anti-heat-shock-protein antibodies and this pregnancy-specific disorder is currently unknown. It could have resulted from an increased sequestration of these antibodies on to heat-shock-protein-expressing tissues or immune complex formation with circulating Hsps. Circulating heat shock protein antibody level is not always raised when the level of the corresponding heat shock protein is elevated in the peripheral circulation or vice versa (Pockley et al. 2000, 2002; Wright et al. 2000). In addition to infections and overexpression/release of Hsps, other factors, such as genetic predisposition, can also regulate the production of these autoantibodies. Our research group reported that a common genetic polymorphism in the promoter region of the interleukin 6 gene and immunoglobulin GM genotypes affect the production of autoantibodies to Hsp60 and Hsp65 (Veres et al. 2002c; Pandey et al. 2004).

The qualitative nature of circulating anti-Hsp antibodies seems also to be important. We have previously observed

that concentrations of complement-activating antihuman Hsp60 antibodies but not total IgG anti-Hsp60 and anti-Hsp65 antibodies are independently associated with high familial risk for coronary heart disease in healthy children (Veres et al. 2002b). Moreover, antibody levels to whole Hsp60 and Hsp65 were not associated with type 1 diabetes mellitus in children, while antibodies to certain epitope regions on Hsp60 were detected in high titers in children with the disease (Horvath et al. 2002). Therefore, concentrations of complement-activating anti-Hsp60 antibodies as well as antibodies against specific Hsp epitopes should be measured to determine the possible role of a true autoimmune reaction towards heat shock proteins in the development of preeclampsia. Nevertheless, it should be taken into account that not only can anti-Hsp antibodies exert harmful effects, but such antibodies can also have protective effects (Wu and Tanguay 2006).

While normal pregnancy appears to be a T helper type 2 (Th2) phenomenon, preeclampsia is characterized by a shift towards a Th1 type immune response (Saito and Sakai 2003). Thus, it is also possible that cellular rather than humoral immune reactions against heat shock proteins expressed in and/or released from the vessel wall or other tissues are involved in the pathogenesis of preeclampsia. Indeed, a cell-mediated autoimmune response to human Hsp60 was found to be associated with a history of

Table 4 Correlation coefficients between clinical characteristics and laboratory parameters of preeclamptic patients and serum anti-Hsp antibody levels

	Serum anti-Hsp60 level	Serum anti-Hsp65 level	Serum anti-Hsp70 level
Age	-0.11	-0.03	-0.08
BMI at blood draw	0.01	-0.13	-0.05
Systolic blood pressure	0.08	0.08	0.03
Diastolic blood pressure	0.04	0.03	-0.01
Gestational age at blood draw	0.01	-0.08	0.14
Gestational age at delivery	-0.14	-0.10	0.14
Fetal birth weight	-0.07	-0.04	0.12
Serum CRP level	0.05	0.06	0.01
Plasma VWF:Ag level ^a	-0.18	0.01	-0.06
Plasma fibronectin level ^a	0.17	0.04	-0.13
Plasma malondialdehyde level ^a	0.23	0.08	0.26
Serum Hsp70 level	-0.01	-0.06	0.11
Serum anti-Hsp60 level		0.59*	0.07
Serum anti-Hsp65 level	0.59*		0.18
Serum anti-Hsp70 level	0.07	0.18	

Significant correlations are in bold

Hsp heat shock protein, BMI body mass index, CRP C-reactive protein, VWF:Ag von Willebrand factor antigen

* $p < 0.001$

^a $n = 67$

spontaneous abortion (Kligman et al. 1998). However, heat shock proteins possess dual immunoregulatory properties, and immune reactivity to endogenous (self-derived) Hsps seems to be anti-inflammatory (Pockley et al. 2008). In the present study, we measured total antihuman Hsp60, anti-mycobacterial Hsp65, and antihuman Hsp70 IgG antibody levels. Although a significant increase has been reported both in IgG1 and IgG2 subclasses in women having a normal pregnancy compared with nonpregnant women (Wilson et al. 2001), anti-Hsp IgG subclass pattern should be investigated in normal pregnancy and preeclampsia in future studies.

In conclusion, anti-Hsp60 and anti-Hsp70 antibodies as naturally occurring autoantibodies are present in the peripheral circulation of healthy pregnant women. Nevertheless, humoral immunity against heat shock proteins was not associated with preeclampsia. Further studies are warranted to explore the role of heat shock proteins and immune reactivity to them in the immunobiology of normal pregnancy and preeclampsia.

Acknowledgements The skillful technical assistance of Szigeti Antalné and the support of Szilvia Walentin, Éva Imreh, and Mónika Kleiber (Central Laboratory, Kútvolgyi Clinical Center, Semmelweis University, Budapest, Hungary) are acknowledged with many thanks. This work was supported by research grants from the Hungarian Scientific Research Fund (NF 72689) and the Faculty of Medicine of the Semmelweis University.

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