Secretion of heat shock -60, -70 kD protein, IL-1β and TNFα levels in serum of a term normal pregnancy and patients with pre-eclampsia development

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Funding Information
Public Health of National Institute of Perinatology “Isidro Espinosa de los Reyes”, México, Grant/Award Number: 212250-3210091

Abstract
The extracellular heat shock proteins (eHsp) family act as molecular chaperones regulating folding, transporting protein and are associated with immune modulation in different physiological and pathological processes. They have been localized in different gestational tissues and their concentration in amniotic fluid and serum has been determined. In the present study, we proposed to determine the concentration of eHsp-60, -70, IL-1β and TNFα in the serum of pregnant patients with 34 weeks of gestation with and without clinical evidences of preeclampsia (PE). Our results indicate significant increase of these markers in patients with PE with respect to healthy pregnant patients without active labor. Finally, the concentration of eHsp-60 and -70 correlated positively with the hepatic dysfunction markers uric acid, lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), and inflammatory IL-1β and TNFα response. In conclusion, our results demonstrate a strong associated between Hsp and marker of hepatic dysfunction.

KEYWORDS
inflammatory cytokine, heat-shock proteins, pre-eclampsia, pregnancy

1 | INTRODUCTION

Pre-eclampsia (PE) is one major obstetrical hypertensive disorder affecting the development of pregnancy, is associated with maternal-foetal morbidity and mortality and intrauterine growth restriction and complicates 8.0% of pregnancies that are treated in the clinical. Although the aetiology of PE is uncertain, the main event is attributed with the inadequate syncytiotrophoblast migration and...
remodelling of maternal spiral arteries results in a deficient maternal blood supply to the placenta, endothelial dysfunction, and systemic inflammatory cytokines. Within this endothelial dysfunction environment provoked by an inadequate syncytiotrophoblast remodelling have been detected extracellular heat-shock proteins (eHsp).

The eHsp are highly conserved molecules that regulate cellular homeostasis, proliferation and differentiation of the immunological cell. The eHsp can be released to extracellular space in response to cellular stress by non-classical protein transport mechanisms. Molvec et al detected in peripheral circulation the 70-kd heat shock protein in normal pregnant women. Several studies have demonstrated the secretion of Hsp in serum of healthy pregnant women and increases in intra-amniotic infection and pregnant women with PE. Here, we determine in maternal serum of pregnancy the concentration of eHsp-60, -70, IL-1β and TNFα in (a) 34-weeks of pregnancy; (b) term in labour; (c) pregnant women with PE; and (d) we determine the correlation between the eHsp-60 and -70 concentrations with hepatic dysfunction markers.

2 | MATERIALS AND METHODS

2.1 | Patients

This study was reviewed and approved by the National Institute of Perinatology Ethics and Research Committees (registration number 212250-3210091). All patients were explained the purpose of the study, and informed consent was obtained. This study included 140 pregnant patients with no obstetric complications and no prior history of PE. Controls group (n = 78) was divided into two categories: patients with 34-weeks of pregnancy (n = 28; the same gestational age as the group of PE) and term in labour (n = 50; with gestational age to term ≥37) defined as dilation of cervical canal (≥4 cm) and uterine contraction sustained. Patients who development clinical data of severe PE that came to the emergency unit (n = 62) was defined according to the American College of Obstetricians and Gynecologists guidelines. PE patients were included in the study previous of the therapeutic treatment. In all cases, blood samples of all patients were taken in only one occasion. The serum was obtained from 5 millilitres of peripheral maternal blood samples and stored at −80°C for the quantification of the eHsp-60, -70, IL1-β and TNFα by specific immunoassay ELISA.

2.2 | Biochemical assays

Commercial ELISA kits were used to measure concentration of eHsp-60, -70, IL1-β and TNFα (R&D Systems, Minneapolis, MN, USA). Standard curve was development from 1.25 to 80 ng/mL, 312.5 to 20,000 pg/mL, 4.0 to 260 pg/mL and 15.0 to 960 pg/mL, respectively, according to commercial manufacturer instructions and has been previously reported by our research group, with a sensitivity of 0.70; 150.0; 2.0 and 5.0 pg/mL, respectively. The values obtained were expressed as pg/mL. Uric acid, creatinine, lactate dehydrogenase (LDH), glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) were measured in a VITROS 4600 Chemistry System (Ortho Clinical Diagnostics, Raritan, NJ) using specific kits. These measurements were determined in the central laboratory of INPerIER.

2.3 | Statistical analysis

Proportional data were analysed using one-way ANOVA, and significant difference between groups were determined by Tukey’s test. The Spearman rank correlation (r) between eHsp and IL-1β, TNFα and clinical parameters were determined. All the assays were independently replicated at least three times, and the data were presented as mean ± SEM. Significant difference was accepted at P value ≤0.05.

3 | RESULTS AND DISCUSSION

Abnormal morphogenesis of the placenta is the main cause for the development of PE, which is associated with spatial and temporal secretion of various markers of endothelial damage and anti-angiogenic factors. It has been demonstrated that molecules secreted into the cell as “alarm system” are eHsp which modulate different cellular process and pathological conditions.

In this study, we found an increase significantly in the term labour group with respect to 34-weeks/pregnancy by 1.3- (P = 0.001), 2.6- (P = 0.004), 5.5- (P = 0.001) and 9.8-fold (P = 0.001) for eHsp-60, and -70, IL-1β and TNFα, respectively (Figure 1). Interestingly, in patients with PE a had a significant increased by 1.44-fold (P = 0.014), and 1.5- (P = 0.003), 5.4- (P = 0.001) and 4.6-fold (P = 0.003) for eHsp-60, and -70, IL-1β and TNFα, concentrations, respectively, with respect to 34-week pregnancy (Figure 1). Furthermore, our results showed significant correlation between Hsp-60 and LDH (r = 0.620; P = 0.0129); GOT (r = 0.521; P = 0.0129), GPT (r = 0.578; P = 0.023), IL-1β (r = 0.699; P = 0.0032) and TNFα (r = 0.720; P = 0.0034) in the PE patients (Table 1). Finally, a significant correlation coefficient between Hsp-70 and uric acid (r = 0.632; P = 0.001), LDH (r = 0.769; P = 0.001); GOT (r = 0.613; P = 0.0023); GPT (r = 0.601; P = 0.02), IL-1β (r = 0.760; P = 0.001) and TNFα (r = 0.690; P = 0.003) was observed in the PE patients (Table 1).

In normal pregnancy, syncytiotrophoblast cells invade and remodel the maternal spiral arteries and different adhesion molecules, angiogenic factors, metalloproteinases and eHsp-27 are detected. The eHsp are spatially and temporally expressed in the human placenta throughout pregnancy. In the first trimesters of pregnancy is mainly of the 27-kd protein; however, this changes to the early stages of labour (37-weeks) reducing the expression of Hsp-27 and increasing the 60 and 70-kd proteins (Figure 1F).11

The biological significance of eHsp-27 activity during the pregnancy in the absence of cell damage suggests that these proteins are involved in cellular protection by reducing the production of molecules associated with oxidative stress, apoptosis, inflammatory...
Interestingly, induce also expression of protein inhibitor α, a negative regulator of the classical nuclear transcription factor-kappa B (NFκB) pathway.10

In this study, we found that in patients with 34-week pregnant, the secretion profile of Hsp-70 was 1.6 ± 0.12 ng/mL (Figure 1). Similar concentrations of Hsp-70 were reported previously by Molverac et al and Fukushima et al5-13 they detected not significant changes in the concentration of Hsp-70 in different stages of pregnancy without active labour.

Additionally, we demonstrated that the secretion profile concentrations increase in patients with active labour with respect to 34-week pregnant (Figure 1). It has been shown that the profile of Hsp in labour activation increased the 70- and reduce the 27-kD protein inducing contraction in the myometrium, secretion of prodegradative
of PE, there was a significant increase in TNF-α patients with 34 weeks and PE development. Indeed, the extracellular function markers and this change in Hsp expression have been determined in other pregnancy pathologies.5,11 Our results demonstrated an increase in Hsp could be used as a simple tool for the detection in the development of PE.

In summary, this study demonstrated that a correlation between eHsp-60 and -70 with respect to hepatic and vascular damage dysfunction markers and this eHsp could be used as a simple tool for the detection in the development of PE.

**ACKNOWLEDGEMENTS**

This work was supported by Public Health of The National Institute of Perinatology “Isidro Espinosa de los Reyes” of México (Grant 212250-3210091 to HFH). We thanks to Secretaria de Salud for supporting the Servicio Social en Investigación studies of ACMC.

**CONFLICT OF INTEREST**

The authors declare that they have no competing interests to disclose.

**AUTHOR CONTRIBUTIONS**

MCAC, ABG and MOC identified patients with clinical evidence of PE and obtained the blood samples from the three study groups. MCAC, EBG, GGL carried out the determination of heat shock protein and inflammatory cytokines. JFA perform the correlation analysis. MCAC, OFH, NFD, AMH and HFH were involved with the study design. HFH obtained the funding and writing the manuscript. NFD and JFA discussed the manuscript in its final form. All authors read and approval the final manuscript.

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**REFERENCES**

1. Haedersdal S, Salvig JD, Aabye M, et al. Inflammatory markers in the second trimester prior to clinical onset of preeclampsia, intrauterine growth restriction, and spontaneous preterm birth. Inflammation. 2013;36:907-913.
2. Walker JJ. Pre-eclampsia. Lancet. 2000;356:1260-1265.
3. Maynard SE, Crawford SL, Bathgate S, et al. Gestational angiogenic biomarker patterns in high risk preeclampsia groups. Am J Obstet Gynecol. 2013;209(53):e1-e9.
4. Stokå P, Wang XN, Dickinson AM. Inducible heat shock protein 70 reduces T cell responses and stimulatory capacity of monocyte-derived dendritic cells. J Biol Chem. 2012;287:12387-12394.
5. Molvarec A, Rigo Jr J, Nagy B, et al. Serum heat shock protein 70 levels are decreased in normal human pregnancy. J Reprod Immunol. 2007;74:163-169.
6. Chaiworapongsa T, Erez O, Kusanovic JP, et al. Amniotic fluid heat shock protein 70 concentration in histologic chorioamnionitis, term and preterm parturition. J Matern Fetal Neonatal Med. 2008;21:449-461.
7. Molvarec A, Rigo Jr J, Lazar L, et al. Increased serum heat-shock protein 70 levels reflect systemic inflammation, oxidative stress and hepatocellular injury in preeclampsia. Cell Stress Chaperones. 2009;14:151-159.
8. American College of Obstetricians, Gynecologists, Task Force on Hypertension in P. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists’ Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122:1122-1131.
9. Osorio-Caballero M, Perdigon-Palacio C, Garcia-Lopez G, et al. Escherichia coli-induced temporal and differential secretion of heat-shock protein 70 and interleukin-1beta by human fetal membranes in a two-compartment culture system. Placenta. 2015;36:262-269.
10. Ferat-Osorio E, Sanchez-Anaya A, Gutierrez-Mendoza M, et al. Heat shock protein 70 down-regulates the production of toll-like receptor-induced pro-inflammatory cytokines by a heat shock factor-

**TABLE 1** Characteristics of the study population and concentration of biochemical parameters

| Parameter                              | PE (n = 62) | CONTROL (n = 28) | Term in labor (n = 50) |
|----------------------------------------|-------------|------------------|------------------------|
| Maternal age (y)                       | 29.9 ± 7.3  | 28.3 ± 8.0       | 22.2 ± 6.3             |
| BMI                                    | 28.86 ± 6.3 | 25.84 ± 5.5      | 26.3 ± 5.2             |
| Systolic blood pressure (mm Hg)        | 149.9 ± 15.3| 106.5 ± 9.8      | 109.8 ± 12.7           |
| Diastolic blood pressure (mm Hg)       | 93.14 ± 9.7 | 67.07 ± 6.6      | 69.2 ± 8.9             |
| Biochemical parameters                |             |                  |                        |
| Uric acid (mg/mL)                      | 5.07 ± 1.5  | 4.16 ± 1.5       | 3.8 ± 0.72             |
| Creatinine (mg/mL)                     | 0.60 ± 0.26 | 0.54 ± 0.08      | 0.52 ± 0.08            |
| LDH (UI/L)                             | 358.43 ± 114| ND               | ND                     |
| GOT (UI/mL)                            | 23.8 ± 24.1 | ND               | ND                     |
| GPT (UI/mL)                            | 22.48 ± 20.4| ND               | ND                     |
| Gestational age, (wk)                  | 34.0 ± 3.9  | 34.0 ± 4.4       | 38.0 ± 2.9             |

Data expresses as mean ± SD. Statistical difference (P < 0.05). Maternal age: (a vs c) and (b vs c); systolic blood pressure: (a vs b) and (a vs c); diastolic blood pressure: (a vs b) and (a vs c); Uric acid: (a vs b) and (a vs c). BMI, body mass index; GOT, glutamic oxalic transaminases; GPT, glutamic piruvic transaminases; LDH, lactate dehydrogenase; ND, no determined; PE, Pre-eclampsia.
1. Constitutive heat shock element-binding factor-dependent mechanism. *J Inflamm*. 2014;11:19.

11. Abdulsid A, Lyall F. Heat shock protein 27 expression is spatially distributed in human placenta and selectively regulated during preeclampsia. *J Reprod Immunol*. 2014;101–102:89-95.

12. Shochet GE, Komemi O, Sadeh-Mestechkin D, et al. Heat shock protein-27 (HSP27) regulates STAT3 and elf4G levels in first trimester human placenta. *J Mol Histol*. 2016;47:555-563.

13. Fukushima A, Kawahara H, Isurugi C, et al. Changes in serum levels of heat shock protein 70 in preterm delivery and pre-eclampsia. *J Obstet Gynaecol Res*. 2005;31:72-77.

14. Abdulsid A, Hanretty K, Lyall F. Heat shock protein 70 expression is spatially distributed in human placenta and selectively upregulated during labor and preeclampsia. *PLoS One*. 2013;8:e54540.

15. Peracoli JC, Bannwart-Castro CF, Romao M, et al. High levels of heat shock protein 70 are associated with pro-inflammatory cytokines and may differentiate early- from late-onset preeclampsia. *J Reprod Immunol*. 2013;100:129-134.

How to cite this article: Álvarez-Cabrera MC, Barrientos-Galeana E, Barrera-García A, et al. Secretion of heat shock -60, -70 kD protein, IL-1β and TNFα levels in serum of a term normal pregnancy and patients with pre-eclampsia development. *J Cell Mol Med*. 2018;22:5748–5752. [https://doi.org/10.1111/jcmm.13824](https://doi.org/10.1111/jcmm.13824)