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Circulating Levels of Heat Shock Protein 70 in Women With Preeclampsia and Healthy Controls

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Background: Pathophysiology of preeclampsia has remained unknown. Excessive maternal systemic inflammatory response to pregnancy along with systemic oxidative stress is one of the underlying theories. Extracellular HSP70 produced from stressed and damaged cells may be involved in the elaboration of maternal systemic inflammatory response.
Objectives: In this regard, we investigated the circulating levels of 70-kiloDalton heat shock protein (Hsp70) in patients with preeclampsia, as well as healthy pregnant women.
Patients and Methods: The study performed during a 6-month-period in Zeinabieh Hospital of Shiraz University of Medical Sciences. We recruited 31 patients with preeclampsia and 31 healthy normotensive pregnant controls (age and ethnicity matched). The serum levels of Hsp70 were measured in all patients and controls and the values were compared between groups. We also compared Hsp70 levels between severe and mild preeclampsia cases.
Results: The mean age of the patients was 27.5 ± 6.3 (range 21 - 42) years, which was comparable to control group 27.7 ± 7.2 (range 19 - 45) years (P = 0.925). The mean systolic and diastolic blood pressure was significantly higher in those with preeclampsia compared to healthy controls (P < 0.001). There was no significant difference between the two study groups regarding the circulating levels of Hsp70 (P = 0.310). We found that the systolic blood pressure was positively associated with gestational age in those with preeclampsia (r = 0.397, P = 0.027). However, there was no correlation between circulating levels of Hsp70 and baseline characteristics as well as blood pressure parameters.
Conclusions: Circulating levels of Hsp70 are not associated with preeclampsia and increased blood pressure in pregnancy. Further studies on the role of heat shock proteins in the pathogenesis of preeclampsia and gestational hypertension is recommended.

Keywords: Heat Shock Protein (Hsp) 70; Preeclampsia; Oxidative Stress; Pregnancy; Placenta

1. Background

Preeclampsia is defined as hypertension accompanied by proteinuria and edema developing after the 20th week of gestation. It is associated with severe maternal and fetal morbidity and mortality (1). The worldwide incidence of preeclampsia has been estimated to be approximately 2% - 10% (2, 3), which accounts for about 50000 maternal deaths each year (2, 4). In spite of the recent advances in the diagnosis and management of preeclampsia, its exact etiology and pathogenesis have remained elusive (5). Several lines of evidence suggest that endothelial cell dysfunction and damage are responsible for the pathogenesis of the disease. In other words, systemic inflammatory response and increased serum levels of oxidative radicals during pregnancy leads to endothelial damage, especially in the placental vessels resulting in dysfunctional vascular response (1, 6, 7). The etiology of preeclampsia is a combination of environmental and genetic factors, which suggest the multifactorial nature of the disease (8, 9).

The heat shock proteins are ubiquitous and phylogenetically conserved molecules, which are categorized into several families and named on the basis of molecular weight. These proteins are produced intracellularly and expressed on the cell surface. The 70-kiloDalton heat shock proteins (Hsp70s) are a family of these proteins, an important part of the cell's machinery for protein folding, and help to protect cells from stress (10, 11). The intracellular inducible Hsp70 can mediate cytoprotective, antiapoptotic, and immune regulatory effects (12). Animal studies have demonstrated that expression of Hsp70 significantly increases in the aorta during the acute hypertension (13). Patients with peripheral and renal arterial diseases have also higher circulating levels of Hsp70 (14).

However, Hsp70 also exists in the peripheral blood of healthy non-pregnant and pregnant individuals but the resource of circulating Hsps has not been completely clarified yet (11, 12). Some data reveal that Hsp70 may be

discharged from viable cells into the extracellular environment exposed to stressful insult. The other studies suggest that Hsp70 can be passively released from damaged, necrotic cells too (12). Placental ischemia, oxidative stress, and maternal systemic inflammatory response, which are major elements in the pathogenesis of preeclampsia, have been shown to induce the expression of Hsp70 (13, 15, 16). It has been demonstrated that the serum level of HSP70 increases in patients with preeclampsia (17-19). Previous reports indicate that serum level of Hsp70 is associated with transient hypertension of pregnancy, preeclampsia, superimposed preeclampsia, the syndrome of hemolysis, elevated liver enzymes, and low platelet count (HELLP syndrome) (20). However, controversy still exists regarding the role of Hsp70 in the pathogenesis of preeclampsia as Livingston and associates (21) found no association between severe preeclampsia and serum levels of Hsp70.

2. Objectives

The present study aimed to compare the circulating levels of Hsp70 in women with preeclampsia to normotensive ones.

3. Patients and Methods

3.1. Study Population

This study performed in Zeinabieh Hospital (a tertiary healthcare center affiliated with Shiraz University of Medical Sciences) from July 2013 to January 2014. The Medical Research Ethics Committee as well as Institutional Review Board (IRB) of Shiraz University of Medical Sciences approved the study protocol. All the patients provided their informed written consents before inclusion in the study. We included 31 pregnant women who were diagnosed with preeclampsia along with 31 health normotensive pregnant women as the control group. All the patients and controls were matched regarding the age, gestational age, ethnicity, parity, and gravidity. Those with multiple pregnancies, Diabetes Mellitus (DM) or gestational DM, autoimmune disorders, chronic hypertension, renal insufficiency, and angiopathies were excluded from the study. We also excluded those mothers who were smokers or consumed alcoholic beverages. Maternal and fetal infections such as chorioamnionitis and syphilis as well as congenital anomalies were further excluded from the study. None of the included patients had ruptured membranes or in the active phase of the labor at the time of inclusion. Control group consisted of 31 consecutively selected healthy normotensive pregnant women attended the outpatient clinics of our center for routine pregnancy follow-ups. Those who developed hypertensive disorders were excluded later.

Preeclampsia was diagnosed according to the criteria of the International Society of the Study of Hypertension in Pregnancy (ISSHP) (22). Preeclampsia was defined as sustained pregnancy-induced hypertension with proteinuria. Hypertension was defined as sustained blood pressure (BP) readings of 140/90 mmHg or higher with readings taking place 6 hours or more apart; or a sustained 15-mm Hg rise in diastolic BP from the first-trimester values, or a 30-mm Hg rise in systolic BP also from the first trimester values. The ISSHP defines proteinuria as a protein concentration of 30 mg/dL or greater (or 1+ on a urine dipstick) in 2 or more random urine specimens collected at least 4 hours apart, and it defines severe PE as a blood pressure reading of 160/110 mm Hg or higher, with either 3+ or 4+ on a dipstick in a random urine sample, or a proteinuria greater than 0.5 g over 24 hours. All the women with preeclampsia and control group recruited for the study were in the third trimester of pregnancy and all of Iranian nationality.

3.2. Study Protocol

All the recruited patients were visited before inclusion in the study and the baseline characteristics, including demographic and obstetrics information were recorded in a questionnaire. A sample of 5 mL of venous blood was withdrawn from the antecubital vein and centrifuged at room temperature at 3000 rpm for 5 minutes. The samples were stored at −20°C until the measurements were performed. Serum Hsp70 levels were measured by using the Enzyme-linked Immunosorbent Assay (ELISA) Kit of R&D Systems (DYC1663 - 2). The intra-assay and inter-assay coefficients of variation (CV) were < 6% for all assays performed.

3.3. Statistical Analysis

Thirty patients were required in each group to have 90% power to detect significant differences between corresponding variables ($P < 0.05$, 2-sided). To compensate for possible useless data, we enrolled 31 participants in each study group. The Statistical Package for Social Science, (SPSS) for Windows, was used for data analysis. Mann-Whitney U test was used to compare the nonparametric data and t test to compare parametric data between the study groups. Data are reported as mean ± SD. A 2-sided P Value < 0.05 was considered statistically significant.

4. Results

Overall, we studied 31 patients with preeclampsia and 31 healthy normotensive pregnant women as the control group. The mean age of the patients was 27.5 ± 6.3 (range 21 - 42) years, which was comparable to control group 27.7 ± 7.2 (range 19 - 45) years. The gestational age was also comparable between the two study groups. The mean systolic and diastolic blood pressure was significantly higher in those with preeclampsia compared to the healthy controls. There was no significant difference between the two study groups regarding the circulating levels of Hsp70 (Table 1).

We found that the systolic blood pressure was positively associated with gestational age in those with preeclampsia ($r = 0.397$, $P = 0.023$). However we found no correlation between circulating levels of Hsp70 and baseline characteristics as well as blood pressure parameters (Table 2).

Table 1. Comparing the Baseline Characteristics and Circulating Level of Hsp70 Between the Two Study Groups [a]

	Preeclampsia	Control	P Value
Age, y	27.5 ± 6.3	27.7 ± 7.2	0.925
Gestational age, wk	35.4 ± 4.1	36.1 ± 2.5	0.426
Systolic BP, mmHg	154.3 ± 10.8	107.5 ± 19.5	< 0.001
Diastolic BP, mmHg	95.6 ± 10.2	72.4 ± 8.6	< 0.001
Hsp70, ng/mL	0.504 ± 0.107	0.763 ± 0.091	0.310

[a] n = 31.

Table 2. Correlations of Circulating Level of Hsp70 With Baseline Characteristics and Blood Pressure in 31 Patients With Preeclampsia

	Correlation Coefficient	P Value
Hsp 70		
Age	- 0.062	0.739
Gestational age	- 0.023	0.904
Systolic blood pressure	- 0.047	0.801
Diastolic blood pressure	0.137	0.462

5. Discussion

The aim of this study was to investigate the role of Hsp70 in the pathogenesis of preeclampsia. We compared the circulating levels of Hsp70 between those with preeclampsia and healthy normotensive pregnant woman. After matching for baseline characteristics, we found that serum levels of Hsp70 are comparable between preeclamptic and normotensive pregnant women. Furthermore, we found no correlation between the systolic and diastolic blood pressures in patients with preeclampsia and the circulating level of Hsp70. These findings suggest that Hsp70 may not play an important role in pathogenesis and etiology of the preeclampsia. Our findings are in agreement with one previous report (21) while inconsistent with other reports (17-20).

Intracellular Hsp70 as a part of cell's machinery for protein folding has an essential function in maintaining cellular hemostasis, but extracellular Hsp70 originated from stressed and necrotic cells can cause innate and adaptive pro-inflammatory immune responses (11). It has been demonstrated that any inflammatory reaction and oxidative stress can induce the expression of Hsp70 (23). As preeclampsia is the result of maternal inappropriate systemic inflammatory response, which leads to oxidative stress and production of oxygen-free radicals (1, 5, 24), heat shock proteins, and especially Hsp70 may play an important role in its pathogenesis (16-20). The exact pathogenesis of preeclampsia remains elusive (5, 7). However, systemic inflammation and oxidative response have been shown to play an important role in it (19, 25).

Previously, Molvarec et al. (20) measured the serum levels of Hsp70 in 142 pregnant women with hypertensive disorders (93 with preeclampsia, 29 with transient hypertension, and 20 with superimposed preeclampsia) and in 127 normotensive, healthy pregnant women. They found higher serum levels of Hsp70 in those with transient hypertension, preeclampsia, and superimposed preeclampsia compared to the healthy controls (20). Likewise, Fukushima et al. (17) also performed a similar study demonstrating the higher serum levels of Hsp70 in patients with preeclampsia and preterm delivery. However, in this study, preterm delivery high risk patients were primarily considered and only 7 patients with preeclampsia were included in this study. Also, Jirecek et al. (18) in a pilot study reported higher serum concentrations of Hsp70 in those with early onset preeclampsia. In addition, Hung et al. (26) found that concentration of Hsp72 is increased in the placental tissues of patients with preeclampsia and this increase was associated with ischemia–reperfusion injury of the placenta. Their study clearly demonstrates the role of oxidative stress and Hsp72 in the pathogenesis of preeclampsia. In contrast, Hnat et al. (27) found no significant difference between the placental concentrations of Hsp70 of patients with preeclampsia and that of normotensive healthy pregnant women. However placental bed biopsies were not investigated in this study. Contrary to all these studies, Livingston et al. (21) found no significant differences between preeclampsia and healthy pregnancy with regard to circulating levels of Hsp70. These results are in line with ours.

Interestingly, although Molvarec et al. (20) found a higher level of Hsp70 in hypertensive pregnant women, there was no significant difference regarding Hsp70 serum levels among transient hypertension, preeclampsia, superimposed preeclampsia, and also between mild and severe preeclamptic patients. They suggest that in these conditions, hemodynamic stress, oxidative stress

(placental or systemic), placental ischemia, ischemia of other organs as well as maternal systemic inflammatory response increase the expression of Hsp70 and cause elevated circulating Hsp70 levels. Indeed, hemodynamic stress (acute hypertension) can stimulate Hsp expression in the vessel wall (13, 16). Inflammatory cytokines, ischemia, and free oxygen radicals are also able to induce Hsp70 expression (15). However, the source of secretion and expression of Hsps in healthy individuals is yet to be identified. We are also not sure whether the circulating levels of Hsp70 show the intracellular concentration. Taking all these together, this point should be kept in mind that the Hsps are not only produced by living and viable cells, but also leaked from necrotized and apoptotic cells (15).

In a recent study, Molvarec et al. (28) found the association of increased serum Hsp70 concentrations in preeclampsia with pro-inflammatory changes in circulating cytokine profile, adhesion molecules, and angiogenic factors suggesting that circulating Hsp70 may play a role in the development of the excessive systemic inflammatory response. On the other hand, Hsp70 can also have anti-inflammatory properties (29) and may play a role in the resolution of inflammation. Molvarec et al.'s finding with respect to the association of serum levels of Hsp70 with IL12p40 (the competitive inhibitor of the bioactive IL-12p70) may indicate an anti-inflammatory effect of circulating Hsp70 in preeclampsia (28).

There are some limitations in our study. The study population was limited and the number of included patients was equal to minimal requirement for having an appropriate power to detect differences. Thus, larger studies are recommended to shed light on the role of Hsp70 in the pathogenesis of preeclampsia.

Circulating levels of Hsp70 are not associated with preeclampsia and increased blood pressure in pregnancy. Further studies on the role of heat shock proteins in the pathogenesis of preeclampsia and gestational hypertension is warranted.

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Authors' Contributions

Study concept and design: Mojgan Akbarzadeh-Jahromi, Fatemeh Sari Aslani; Acquisition of data: Mojgan Akbarzadeh-Jahromi, Zahra Daneshyar, and Nasrin Asadi; Analysis and interpretation of data: Mojgan Akbarzadeh-Jahromi, Fatemeh Sari Aslani, and Zahra Daneshyar; Drafting of the manuscript: Mojgan Akbarzadeh-Jahromi; Administrative, technical, and material support: Hamid Reza Zare; Statistical analysis: Mojgan Akbarzadeh-Jahromi and Zahra Daneshyar; and Critical revision of the manuscript for important intellectual content: All Authors.

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