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EVALUATION OF HSP 70/90 LEVELS IN PREGNANT WOMEN WITH MILD PREECLAMPSIA

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Abstract

Preeclampsia is still one of the leading causes of maternal and perinatal mortality and morbidity worldwide.). Overexpression of HSPs is induced by many types of cell stress, such as oxidative stress, inflammation, and toxic compounds. HSP 70 and HSP 90 have important roles in the regulation of the cell's life process, cell functions, and continuousness of immunity. In this study, we aimed to investigate HSP 70/90 expressions in mild preeclampsia groups. 30 pregnancy were included in the study, 15 were control and 15 were mild preeclampsia at Gaziantep University Şahinbey Research and Application Hospital Department of Obstetrics and Gynecology. As the control group, umbilical tissues taken from 15 women who did not have any metabolic disease were used. Mild preeclampsia (MP) was considered if systolic blood pressure was greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg and proteinuria more than 4g in a 24-hour urine sample and their umbilical tissues were collected to examine them in experimental stages. The umbilical cords of pregnant women were taken during delivery for immünohistochemia and western blot. HSP 70/90 antibodies were examined using western blot. Also, HSP 70/90 expression was evaluated by immunohistochemistry method. Western blot and immunohistochemical methods were carried out in experimental stages. The western blot and immunohistochemia analyses showed that the expressions of the proteins HSP 70/90 were significantly higher in the umbilical cords of women with mild preeclampsia than in the umbilical cords of control women. The relationship between HSP 70/90 and PE is stil controversial. These data suggest that upregulated HSP 70/90 levels were probably associated with increased apoptosis in the umbilical tissues of PE patients and may contribute to the pathophysiology of PE.

Key Words: Mild Preeclampsia, Pregnancy, Umbilical Tissue

1. INTRODUCTION

Hypertensive syndromes that occur during pregnancy, especially preeclampsia (PE), cause real risk and significant impact on maternal and child health indicators (1). PE, which is a multi-systemic pregnancy disease and occurs after the 20th gestational week, is a problem characterized by hypertension and proteinuria (2, 3). PE was still among the leading causes of maternal and perinatal mortality and morbidity (4, 5). Although its etiology is not fully understood, The insufficiency of trophoblast invasion and changes in spiral arteries in the placental bed causes insufficient perfusion of the placenta and thus hypoxia. In other words, PE is associated with weakened or impaired trophoblast infestation. Inadequate spiral artery differentiation and trophoblast spreading were reported in patients with PE (6, 7).

Heat shock proteins (HSPs) are main components of the cellular stress response to decrease injury, accelerate regeneration, and maintain homeostasis (8). Overexpression of HSPs is induced by many types of cell stress, such as oxidative stress, inflammation, and toxic compounds (9). The hypoxic condition compels trophoblast and vascular endothelium cells to express more HSP70. Although the induced HSP70 can protect cells from stress-induced damage by preventing protein denaturation and/or by repairing such damage, HSP70 binding sites on inflammatory cells, could promote leukocytes adhesion and invasion to placental tissue (10). HSP90 has an important role in the regulation of the cell's life process, cell functions, and continuousness of immunity (11).

In the studies conducted so far, HSP70/90 levels in the placentas of pregnant women with preeclampsia have been examined very little, and also there is no study on this proteins expression in the umbilical tissues. In this study, we aimed to investigate the levels of HSP70/90 in the umbilical tissues of pregnant women with severe preeclampsia using both immunohistochemistry and western blot analysis method and to contribute to the literature in preeclampsia pathology.

2. MATERIAL AND METHODS

The study protocol was accepted by the Gaziantep University's Human Ethics' Committee with number 2014/305. This study was carried out on umbilical cords taken from 30 women (15 mild preeclampsia (MP) and 15 Control) aged between 25-30 who applied to Gaziantep University Şahinbey Research and Practice Hospital, Department of Obstetrics and Gynecology after delivery. The study was conducted in Gaziantep University Faculty of Medicine, Histology-Embryology Department Laboratory.

2.1. Tissue Tracking

Umbilical cord fragments taken after birth were fixed in 10% neutral formalin solution for 10 days. It was divided into pieces of suitable size for tissue follow-up and taken into lidded cassettes. the tissues were washed in running water for 1 hour to clear the fixative solution. Water removed by passing through increasingly graded alcohols. Paraffin blocks were prepared by paraffin inclusion after transparentization with xylene. 5 micrometer thick sections were cut from the blocks using a Leica RM 2145 model microtome and prepared for staining.

2.2. Immunohistochemical Analysis

The sectioned praparates were passed through xylene series after waiting at least two hours in a 65-70 degree oven. Then it was passed through the decreasing alcohol series, placed in distilled water and placed in a citrate buffer, and then put in the microwave for 10 minutes for antigen recovery and cooled at room temperature for 10 minutes. Ultraviole V block was instilled after H2O2 was taken. HSP 70/90 primary antibodies were dropped and kept at +4 overnight. The next morning, it was taken into the secondary antibody, streptavidin HRP was dropped, and after the DAB solution, it was passed through the alcohol and xylene series again, and finally it was covered with a coverslip using entellan. Image J program was used for immunoreactivity (14).

2.3. Western Blot Analysis

Umbilical tissues were homogenized and lysed in radioimmunoprecipitation assay buffer supplemented with 1 mM phenylmethanesulfonyl fluoride for 1 h and then centrifuged at 15 000 r.p.m. for 30 min at 4 °C. The protein concentration was measured using a BCA protein assay kit. Equal amounts of protein (40 μg per lane) were separated on 10% SDS–polyacrylamide gels and then transferred onto polyvinylidene fluoride membranes (Millipore, Bedford, MA, USA). The membrane was blocked in Tris-buffered saline containing 5% nonfat milk powder for 1 h and then incubated overnight with HSP70/90 antibodies, each diluted in Tris-buffered saline/5% nonfat milk powder; the samples were subsequently incubated with an antibody against beta actin (β-actin) as a loading control. The membrane was washed three times with Tris-buffered saline containing Tween-20 and then incubated with horseradish peroxidase-conjugated anti-rabbit IgG (1:1500) for 1 h at room temperature. Proteins were detected by enhanced chemiluminescence reagents. The levels of expression of the proteins were analyzed using ImageJ software.

3. Statistical Analysis

All statistical analyses were carried out by using GraphPad Prism version 7.00 for Mac, GraphPad Software, La Jolla, California, USA. D'Agostino Pearson omnibus test was used to identify the normal distribution of the data. In the case of normal distribution, quantitative variables were compared using one-way analysis of variance (ANOVA) and Tukey's posthoc test. Kruskal Wallis test and Tukey's post-hoc test were used for comparing the quantitative with the abnormal distribution. The data were expressed as the mean of normalized data±standard deviation of the mean. $p<0.05$ was considered as statistically significant.

4. RESULTS

4.1. Characteristics of The Working Groups

In our study, no statistically significant difference was observed between the ages of normal and mild pregnant women ($p>0.05$). However, when we looked at gestational age at birth (weeks), fetal birth weight, systolic blood pressure (mm Hg) and diastolic blood pressure (mm Hg), it was observed that there was a statistically significant difference between the two groups ($p<0.05$). Also, Also, when we looked at uric acid and urine protein in the urine of these two groups, it was observed that there was a significant increase in the mild preeclampsia group ($p<0.05$) (Table 1).

Table 1. Characteristics Of Working Groups

	Control	Mild Preeclampsia	*p*
Age	29.3 ± 1.2	29.8 ± 16	0.692
Gestational age at birth (weeks)	39.2 ± 1.1	29.3 ± 1.9	0.015
Systolic blood pressure (mm Hg)	115.5 ± 6.7	142.9 ± 7.8	0.001

Diastolic blood pressure (mm Hg)	72.3 ± 4.8	98.5 ± 6.9	0.001
Uric acid	323.5± 92.1	446.7± 77.8	0.001
Urine protein/24 h	-	4.3 ± 1.7	0.001
Fetal birth weight	3199.2± 300.2	1995.4± 426.1	0.001

4.2. Immunohistochemical findings

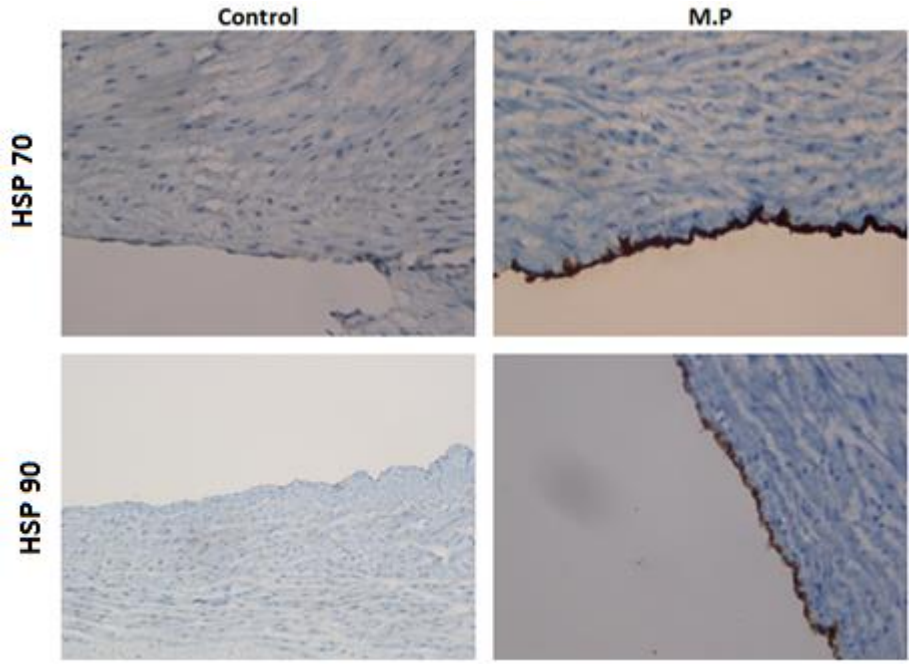
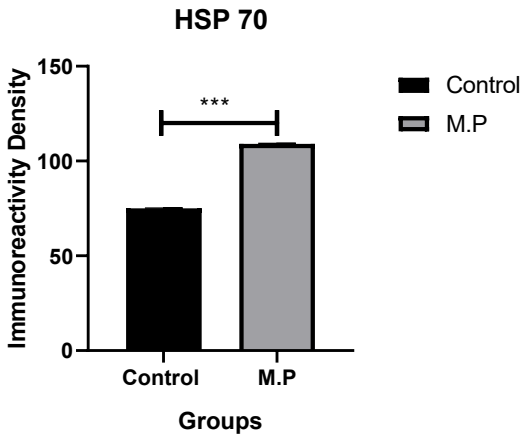


Figure 1. HSP 70/90 immunohistochemistry staining.



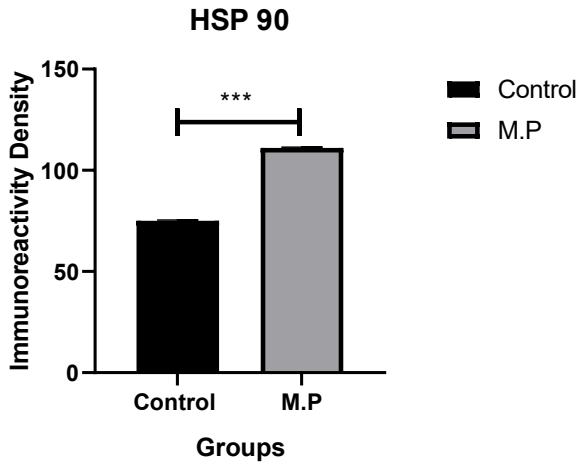


Figure 2. HSP 70/90 immunoreactivity results.

Immunohistochemical staining was performed using the avidin–biotin method to determine the umbilical tissue expressions of HSP70/90. Immunohistochemical examinations demonstrated the presence of HSP70/90 immunostaining in the vascular endothelium. HSP70/90 immunoreactivities were considerably increased in mild preeclampsia (MP) group ($p<0.05$). Figure 1 show the HSP70/90 expression in MP group.

4.3. Western blot findings

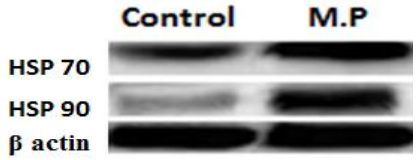
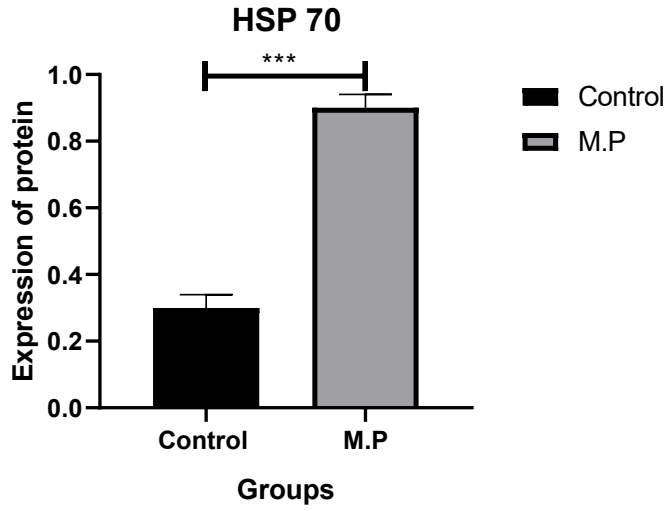


Figure 3. HSP 70/90 protein band view



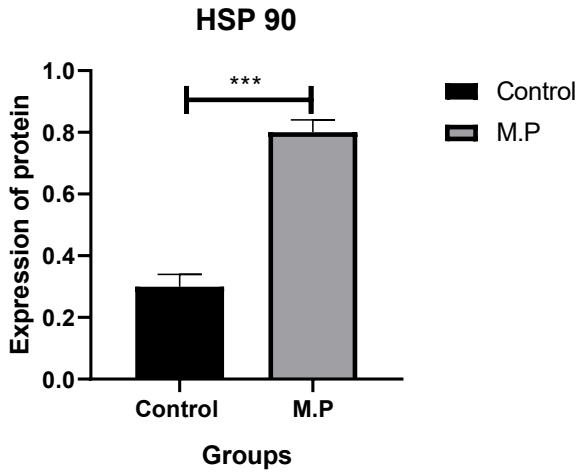


Figure 4. HSP 70/90 expressions of proteins.

β actin was used as a loading control in western blot analysis. According to the results of western blot, a statistically significant increase in the HSP 70/90 expression band was found in mild preeclampsia group (p<0.05). Figure 3,4 show the HSP 70/90 expression.

5. DISCUSSION

Preeclampsia is a common pregnancy specific disease with potential adverse maternal and neonatal outcome, that affects 3–5% of all pregnancies. Maternal complications; It covers a wide spectrum from abruptio placenta, intracranial hemorrhage, liver failure and kidney failure to death. Fetal complications include intrauterine growth retardation, premature birth, and perinatal asphyxia (12, 13). In preeclampsia physiopathology; As a result of insufficient uteroplacental vascularization, sufficient blood supply cannot be provided to the developing fetus and fetoplacental hypoxia develops (14). While the etiology is not fully understood, it is clear that preeclampsia is a complex obstetrical syndrome that is uniformly associated with maternal vascular dysfunction, and impairments in nitric oxide signaling likely play a key role in driving disease progression and severity (15).

Godhamgaonka et al. reported that there is no significant difference in age between preeclampsia and healthy pregnant women (16). Similarly, Gupta et al. reported that there is no significant difference in terms of age and height between pregnant women with preeclampsia and healthy pregnant women (17).

When we look at the age rates of the groups included in the study; There was no significant difference between pregnant women with mild preeclampsia and healthy control group. In the results we found, it was seen that systolic and diastolic blood pressures of mild preeclamptic pregnant women were significantly higher than normal pregnant women. In addition, we found that mild preeclamptic pregnant women had significantly higher uric acid and urine protein compared to normal pregnant women. These results were similar to previous studies.

Heat shock proteins expressed in cells and tissues have important functions especially in stress situations (18). Some HSPs are expressed constitutively while others are induced by a range of damaging insults including heat shock, oxidative stress, hypoxia, ischemia and physical injury (19). . The inducible HSP70/90 are one of the best studied HSPs (11, 20). In addition, it has been reported that HSPs play significant signaling roles in the extracellular microenvironment. HSP70

has been demonstrated to be released from cells after acute stress as well as being secreted after exposure to a number of stimuli (14). It has been shown that the HSP70 serum concentration in preeclampsia is increased in comparison to normal pregnanc (10, 21, 22). Romão-Veiga et al. reported in their study that HSP 70 increased in the serum of preeclamptic women (23). Sheiki et al. reported in their study that HSP 70 increased in the placental tissues of preeclamptic pregnant women (10). Saghafi et al., in their study on preeclamptic pregnancy, showed an increase in HSP 70 expression in the serum of women with preeclampsia compared to normal pregnant women (19). Abdulsid et al. reported an increase in the HSP 70 expression level in the placentas of women with preeclampsia in their study of preeclamptic women (24). Peracoli et al. reported in their study that increased HSP 70 expression in women with preeclampsia may be associated with proinflammatory cytokines (25). Molvarec et al. reported that increased HSP 70 in the serum of women with preeclampsia may be associated with circulating cytokines, chemokines, adhesion molecules (26). Similar to previous studies, we found that there was an increase in HSP70 expression in umbilical cord of pregnant women with mild preeclampsia. There are very few studies on HSP 90 in women with preeclampsia in studies conducted so far. In their study, padmini et al. reported that there was an increase of HSP 70 and HSP 90 in the placental tissues of women with preeclampsia (27).

As metabolically active tissues vital to the maintenance of pregnancy, placental tissue and umbilical cord experiences overstress and are more susceptible to ROS mediated apoptosis in preeclampsia. The increased expression of HSP70 and 90 under such conditions favor maintenance of pregnancy by their antiapoptotic function. In this study, we evaluated HSP 70 and HSP 90 expressions in the umbilical ligament tissues of mild preeclamptic pregnants and healthy pregnant women by both immunohistochemical and western blot methods. According to the results of both experiments, we found that HSP 70/90 increased in pregnant women with mild preeclampsia. The physiological and pathological significance of this remains to be elucidated.

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