Original Articles

Heat shock protein 27 in the placentas of women with and without severe preeclampsia

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Summary

Background: Although not fully understood, heat shock proteins (HSP) are well known stress response proteins. The purpose of this analysis was to determine whether staining for HSP27 was different between placentas from pregnancies complicated by severe pre-eclampsia with intrauterine growth restriction (IUGR) as compared to controls.

Methods: Sterile placental tissue was collected from ten women whose pregnancies were complicated by severe preeclampsia with IUGR and from ten women with uncomplicated by severe pre-eclampsia with IUGR and from ten women with uncomplicated term pregnancies. The tissue was then stained for HSP27.

Results: The median age of the patients was 27 years (mean 27, range 17-37). The median estimated gestational age at delivery was 38 weeks (mean 37, range 29-41). Overall 12 of 20 placentas stained positively for HSP27 (nuclear and/or cytoplasmic). Eight of ten placentas from women with pre-eclampsia and IUGR stained positively for HSP27 ($p = 0.046$).

Conclusion: HSP27 staining of the placenta is twice as common in patients with severe preeclampsia as compared to patients with normal term gestations. These preliminary results warrant the inauguration of a similar but larger study to examine the significance of these findings.

Key words: Preeclampsia; Placenta; Peripheral blood.

Introduction

In 1974, the discovery of a high expression of stress response or heat shock protein (HSP) was found to accompany chromosomal puffing. Previously, this puffing was described as the heat shock response seen in Drosophilia after applied heat stress [1, 2]. Further studies, during the 1970s and 1980s demonstrated that the same rapid synthesis of a small group of highly conserved HSPs occurs in most organisms in response to heat shock and various other stressors including exposure to heavy metals, oxidants, tissue trauma or ischemia, inflammation, and anti-neoplastic drugs [3, 4]. Less stressful conditions such as the normal cell cycle, cell differentiation, hormonal stimulation, and stimulation of proto-oncogenes also elicit and HSP response. HSPs apparently assist the cell in surviving or resisting stressful conditions by an incompletely understood mechanism [3, 4]. There are suggestions that HSPs are important in protein assembly, immunity, and autoimmunity [5]. The major functions of stress induced HSPs seem to be prevention from protein aggregation, misfolding of denatured cellular proteins, and renaturation of cellular proteins [6]. The role of HSPs in cellular proliferation and drug resistance makes these proteins particularly intriguing for cancer research or for other metabolically active tissues like placenta.

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The purpose of this pilot study was to determine whether staining the stress response protein HSP27 was different in the placentas of term, healthy controls as compared to its appearance in the placentas of women whose pregnancies were complicated by severe preeclampsia with intrauterine growth restriction (IUGR).

Patients and Methods

Tissue from the placentas of ten consecutive women with severe preeclampsia (defined as 24-hour urine protein > 5 g, systolic blood pressure > 160 mm Hgb, diastolic blood pressure > 100 mm Hgb) were collected immediately after delivery of the placenta in a sterile fashion and were placed directly into neutral buffered formalin. To be eligible, the pregnancy also had to be complicated by IUGR (estimated fetal weight below the 10th percentile for gestational age) that was judged to be secondary to the preeclampsia by the maternal fetal medicine consultant involved with the case (A.K.H.).

Placenta samples were collected in a similar fashion from control patients immediately after delivery of the placenta and were placed directly into neutral buffered formalin. To be eligible as a control, the patient's pregnancy could not be complicated by preterm delivery (below 37 weeks' estimated gestational age), preeclampsia, or chronic hypertension, fetal anomalies, diabetes mellitus, or chorioamnionitis. The next patient delivering and meeting these criteria after each study case was considered a control case.

Five-micrometer sections of placenta were cut and placed on slides. After fixation, the slides were rinsed with PBS. Endogenous peroxidases were blocked with 1% hydrogen peroxide in

methanol for 10 min. The anti-HSP27 monoclonal antibody NCL-HSP27 (Novocastra Lab, U.K.) at a 1:30 working dilution was added to the slides and incubated 90 minutes at room temperature. After being rinsed twice with PBS, biotinylated anti-mouse IgG and avidin-biotin complex (Vector Laboratory, Burlingame, CA, ABC kit 6102) were used for immunohistochemical staining. The slides were developed with 3, 3 diaminobenzidine tetrahydrochloride (Polyscience Inc., Warinton, PA) and counter-stained with 0.5% methyl green in 0.1 M sodium citrate buffer (pH 4.0) for one minute. Sections were dehydrated with 100% isopropanol and mounted with Permount. Light microscopy was used to determine whether HSP27 IHC staining was positive or negative in either the nucleus or cytoplasm.

Statistics, including χ^2 were performed utilizing SPSS for Windows verion 9.0 (Chicago, IL).

Results

Twenty patients were included in the study. Ten patients had severe preeclampsia with IUGR, and ten patients had uncomplicated term pregnancies. The average age of the patients was 27 years (median 27, range 17-37). There was no difference in the age of women with severe preeclampsia (mean 26 years old) as compared to the women with uncomplicated pregnancies (mean 28 years old) ($p = 0.45$). The average number of pregnancies was two (median 2, range 1-8). There was no difference in the parity for women with severe preeclampsia (2 pregnancies) as compared to the women with uncomplicated pregnancies (2 pregnancies) ($p = 0.83$). The average estimated gestation age (EGA) at delivery for the entire cohort was 37 weeks (median 38, range 29-41). Deliveries in patients with severe preeclampsia (33 weeks) occurred earlier than deliveries in patients with uncomplicated pregnancies (40 weeks) ($p < 0.001$).

The results of placental staining for HSP27 are listed in Table 1. Cytoplasmic staining for HSP27 was twice as common in women with severe preeclampsia as compared to the placentas of women with normal term deliveries ($p = 0.046$).

Table 1. — *Placental HSP27 staining in patients with and without severe preeclampsia.*

	Percentage of placentas demonstrating nuclear HSP27 staining	Percentage of placentas demonstrating cytoplasmic HSP27 staining	Percentage of placentas demonstrating both nuclear and cytoplasmic HSP27 staining
Severe preeclampsia			
Present	30%	80%	30%
Absent	30%	40%	10%

Discussion

The presence of heat shock proteins in the placenta was first documented in 1995 [7]. Divers and collagues discovered no difference in placental HSP response in preterm deliveries as compared to term deliveries. Furthermore, they reported no difference between vaginal and cesarean deliveries in placental HSP response. HSPs, especially HSP60, HSP70, and HSP90, appear to remain constant throughout the third trimester in pregnancy, unless another factor adds additional stress to the pregnancy [7]. While Ziegert *et al.* found the level of HSPs to be constant in placentas throughout the third trimester, they also noted that circulating HSP-antibody complexes appeared to be absent in some women with preterm labor [8].

Li *et al.* studied HSPs in third trimester pregnancies [9]. HSP27 was found to be expressed in both the vascular smooth muscle of the umbilical cord as well as in the placenta. HSP27 was found to be present in both the vascular smooth muscle of the umbilical cord and in the placenta, however, its expression was much higher in the umbilical vessels than in the placenta. Additionally, Li and associates noted no correlation between the method of delivery, APGAR score, gestational age at delivery, fetal outcome, or cord pH and the magnitude or distribution of the HSP response.

In contrast, Shah and colleagues discovered no evidence of HSP27 response in third-trimester placentas [10]. They reported, however, that HSP60, HSP70, and HSP90 were present in placentas throughout the third trimester.

In the current group of patients, eight of ten patients with severe preeclampsia had HSP27 staining of the placentas. Since Li and colleagues found that EGA, mode of delivery, and fetal outcome did not affect HSP staining magnitude or distribution [9], an argument can be made that the difference in staining of HSP27 found in this cohort of patients was due to the added physiologic stress of severe preeclampsia. Since HSPs can be measured in the peripheral blood [11], if larger studies still show differential staining, HSPs may potentially become a marker for preeclampsia.

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