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Journal of Reproductive Immunology 74 (2007) 163–169

JOURNAL OF REPRODUCTIVE IMMUNOLOGY

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Serum heat shock protein 70 levels are decreased in normal human pregnancy

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Received 10 October 2006; received in revised form 14 November 2006; accepted 19 December 2006

Abstract

Heat shock proteins (Hsps) are primarily known to be intracellular proteins with molecular chaperone and cytoprotective functions. However, Hsp60 and Hsp70 have been found in the serum and plasma of healthy non-pregnant individuals. We aimed to compare serum Hsp70 concentrations in healthy pregnant women with those of healthy non-pregnant women and to determine factors influencing serum Hsp70 levels in normal pregnancy. One hundred and seventy six healthy pregnant women with uncomplicated pregnancies (age, 17–44 years; gestational age, 20–41 weeks) and 81 healthy, age-matched non-pregnant women (age, 22–40 years) were enrolled in this cross-sectional study. Serum Hsp70 concentrations were measured using an enzyme-linked immunosorbent assay, and were significantly lower in healthy pregnant women than in healthy non-pregnant women (median (25–75 percentile): 0.29 (0.20–0.35) ng/ml versus 1.27 (0.86–1.72) ng/ml; $p < 0.001$). In healthy pregnant women, there was a statistically significant negative correlation between maternal age and serum Hsp70 concentration (Spearman $R = -0.35$; $p < 0.001$) and a significant positive correlation between gestational age and serum Hsp70 level (Spearman $R = 0.35$; $p < 0.001$). The capacity of extracellular Hsp70 to elicit innate and adaptive proinflammatory immune responses might be harmful in pregnancy and lead to immune rejection of the fetal semi-allograft. We hypothesize that decreased circulating Hsp70 levels are due to unknown regulatory mechanisms aimed at maintaining immune tolerance in pregnancy. In conclusion, serum Hsp70 concentrations are decreased in normal human pregnancy; however, further studies are needed to explain the observed differences between pregnant and non-pregnant women.

Keywords: Heat shock protein 70; Serum concentrations; Pregnancy; Immune tolerance; Maternal age; Gestational age

1. Introduction

Heat shock proteins (Hsps) are ubiquitous and phylogenetically conserved molecules, which suggest their functional importance. Hsps are classified traditionally on the basis of their molecular weight (Prohaszka and Fust, 2004). They are usually considered to be intracellular proteins and have molecular chaperone and cytoprotective functions (Hightower, 1991). However, Hsps can be expressed also on the cell surface (Multhoff and Hightower, 1996; Soltys and Gupta, 1997). In addition, Hsp60 and Hsp70 have been reported to be present in the serum and plasma of healthy non-pregnant indi-

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doi:10.1016/j.jri.2006.12.002

viduals, but the source of circulating Hsps has not been determined yet (Pockley et al., 1998, 1999; Lewthwaite et al., 2002). Increasing evidence suggests that Hsp70 may be released from viable cells into the extracellular environment within exosomes by a non-classical (endoplasmic reticulum-Golgi-independent) protein transport mechanism requiring intact lipid rafts and this mechanism can be induced by certain cytokines (Bausero et al., 2005). Nevertheless, it may be released also as free Hsp70 (Asea, 2005). Furthermore, Hsp70 might be discharged from necrotized (and, to lesser extent, from apoptotic) cells (Prohaszka and Fust, 2004).

Serum Hsp70 concentrations have been studied in normal and pathological pregnancies, and elevated serum Hsp70 levels found to be associated with preeclampsia and preterm delivery (Jirecek et al., 2002; Fukushima et al., 2005). However, a contradictory study also exists (Livingston et al., 2002). We have demonstrated previously that serum Hsp70 concentrations are elevated in transient hypertension of pregnancy, in preeclampsia and in superimposed preeclampsia (Molvarec et al., 2006). Moreover, serum Hsp70 levels are significantly higher in patients with HELLP syndrome compared with severe preeclamptic patients without HELLP syndrome (Molvarec et al., 2007). Nevertheless, little is known about circulating Hsp70 levels in normal human pregnancy.

Jirecek et al. (2002), as well as Fukushima et al. (2005), determined serum Hsp70 concentrations with an enzyme-linked immunosorbent assay, whereas Bloshchinskaya and Davidovich (2003) measured Hsp70 levels in blood plasma using an immunoblotting procedure. Their results regarding changes in circulating levels of Hsp70 during normal pregnancy are controversial. Fukushima et al. (2005) did not find significant differences in serum Hsp70 concentrations between the three trimesters. Conversely, in the study of Jirecek et al. (2002), serum Hsp70 levels tended to decrease, whereas, in that of Bloshchinskaya and Davidovich (2003), Hsp70 concentrations in blood plasma tended to increase with advancing gestation. In addition, they examined only the effects of gestational age on circulating Hsp70 levels in normal pregnancy.

Bloshchinskaya and Davidovich (2003) as well as Fukushima et al. (2005), compared circulating Hsp70 concentrations between healthy pregnant and non-pregnant women and observed non-significantly higher Hsp70 levels in pregnant compared with non-pregnant women. However, in their studies, pregnant women with gestosis and preclinical gestosis or preterm delivery high-risk patients were primarily investigated and there were only 16 and 7 healthy non-pregnant women enrolled, respectively.

Therefore, our aim in the present study was to compare serum Hsp70 concentrations in healthy pregnant compared with healthy non-pregnant women in a larger cross-sectional study and to determine factors influencing serum Hsp70 levels in normal human pregnancy.

2. Materials and methods

2.1. Study patients

One hundred and seventy six healthy pregnant women with uncomplicated pregnancies (aged between 17 and 44 years; gestational age between 20 and 41 weeks) and 81 healthy non-pregnant women (aged between 22 and 40 years) were enrolled in this cross-sectional study. Patients were recruited from the Department of Obstetrics and Gynecology in the Kútvölgyi Clinical Center, the 1st Department of Obstetrics and Gynecology and the 3rd Department of Internal Medicine at the Semmelweis University, Budapest, Hungary. Pregnant women were consecutively selected from a group of healthy pregnant women in outpatient clinics who were undergoing routine blood tests, and were excluded if they later developed any pathological condition. Non-pregnant women were matched to pregnant women on the basis of age, and were selected accordingly from a group of 323 healthy non-pregnant women aged between 22 and 79 years. The women were fasting, none of the pregnant women were in active labour, and none had rupture of membranes. All women were of Caucasian origin. Pregnant women with multifetal gestation, fetal infection and fetal congenital anomaly were excluded from the study. One hundred and twenty seven of the 176 healthy pregnant women served as a control group in an earlier study (Molvarec et al., 2006).

The study protocol was approved by the Regional, Institutional Committee of Medical Ethics at the Semmelweis University, and each patient provided written informed consent.

2.2. Biological samples

Maternal blood samples were collected from an antecubital vein and centrifuged at room temperature at $3000 \times g$ for 10 min. Serum was stored at $-80\,^{\circ}C$ until analysis was performed.

2.3. Serum Hsp70 analysis

Soluble Hsp70 level was measured using an R&D Systems (Minneapolis, MN, USA; Cat. No. DYC1663E)