

Serum Levels Evaluation of Heat Shock Protein70 during Gestation and Fetal Birth Weight in Asthmatic Women of Thi-QAR Province, Iraq

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Abstract

Asthma is a multifactorial inflammatory disease of the upper airways and it's one of the most common diseases complicating pregnancy and represents a risk factor for several maternal and perinatal complications. The natural history of asthma is known to change in pregnancy, but very few data are available in the terms of pathomechanism of this change during gestation. Circulating heat shock protein 70 (Hsp70) levels are decreased in healthy pregnancy, which might reflect physiological immunotolerance. The aim of our study was to determine the serum levels of Hsp70 in asthmatic women during gestation. Fifty pregnant women with bronchial asthma and 50 healthy pregnant women matched for maternal and gestational age were involved in this case-control study. Serum Hsp70 levels were measured using the Bioassay ELISA Kit. In asthmatic pregnant women, an increase of serum Hsp70 levels was observed compared to healthy pregnant women (median 18–75 percentile): 0.46 ng/ml versus 0.16 ng/ml $p < 0.001$). Fetal birth weight of asthmatic mothers was significantly smaller than of healthy controls, but in the normal range (3,580 g versus 3,230 g, $p > 0.05$). A statistically significant negative correlation between maternal age and serum Hsp70 concentrations ($p > 0.05$) and a significant were detected in healthy pregnant women. This study proves an elevation of circulating Hsp70 levels during asthmatic pregnancy compared to healthy pregnant women.

Keywords: Asthma , Pregnancy , Heat shock protein 70 (Hsp70), Fetal birth weight.

Introduction

Heat shock proteins (Hsps) are ubiquitous and phylogenetically conserved molecules, which indicate their functional importance. They are usually considered to be intracellular proteins with molecular chaperone and cytoprotective functions [1]. However, Hsp 60 and Hsp70 have been shown to be present in the serum and plasma of healthy nonpregnant women [2,3] and serum Hsp70 levels are lower in healthy pregnant women than in healthy nonpregnant women [4]. The role of Hsp downregulation in normal pregnancy is not known, but we may be decreased circulating Hsp70 levels are due to mechanisms that maintain immune tolerance in pregnancy. In that study, circulating Hsp70 levels increased with advancing gestational age and decreased with increasing maternal age. The age-related decrease in Hsp70 levels observed in healthy pregnant women is in accordance with findings in nonpregnant subjects, and it might be explained by reduced ability of cells to respond to stress with increasing age [5,6,7]. Increased circulating Hsp 70 levels during pregnancy may be

associated with maternal and fetal complications. Several previous studies reported elevated serum Hsp 70 levels during pregnancy associated with preeclampsia, preterm delivery, and HELLP syndrome [8,9,10,11,12,13]. However, contradictory data also exist [14].

Asthma is among the most common, potentially serious medical problems that complicate pregnancy. The most commonly reported maternal and fetal complications in asthmatic pregnant women are very similar to those with high Hsp 70 levels: preeclampsia, preterm delivery, and infants with low birth weight or intrauterine growth restriction [15,16,17]. A very recent study by Breton and coworkers of a database cohort of 13,100 pregnant asthmatics reported a 35% increased risk of perinatal mortality in the pregnancies of women with asthma, which may be partly explained by a higher rate of low birth-weight babies and preterm delivery [18]. The confounding factors contributing to this increased perinatal mortality might be maternal obesity and smoking, as well as uncontrolled asthma [19].

According to their results, the incidence of preterm delivery was significantly higher among patients with inadequate asthma symptom control during the first part of pregnancy compared with patients with adequate asthma control, and patients who were hospitalized for asthma during pregnancy had a higher incidence of preterm delivery compared with asthmatic women without a history of hospitalization, so there may be a risk for preterm delivery posed by poorly controlled maternal asthma [20]. Maternal asthma is also known as a risk factor for the development of asthma in children [21,22,23]. The natural history of asthma is known to change in pregnancy, but very few data are available in terms of the pathomechanism of this change during gestation [24]. Culminating proliferation of circulating interferon- γ and interleukin-4 positive T-lymphocytes associated with uncontrolled asthmatic inflammation, which may potentially impair maternal airway symptoms as well as fetal development [25]. It may be plausible that hypothesized altered Hsp70 levels in asthmatic pregnant women may play a role in decreased immunological tolerance that characterizes asthmatic pregnancies and result in perinatal and maternal complications.

The aim of our study was to detect the circulating levels of Hsp70 during gestation and Fetal birth weight in asthmatic women.

Methods

Subjects

Fifty pregnant women with bronchial asthma and 50 healthy pregnant women matched for maternal and gestational age were involved in this case-control study. The study participants were enrolled in the Bent –Al Huda teaching Hospital (Nasiriyah) Iraq.

All women were resided in the same geographic area in Thi –Qar province, IRAQ. Exclusion criteria were multifetal gestation, hypertensive disorders, diabetes mellitus, autoimmune disease, angiopathy, renal disorder, maternal or fetal infection, and fetal congenital anomaly. The women were fasting, none were in active labor, and none had rupture of membranes. All patients were prescribed inhaled corticosteroids. Fetal growth restriction was diagnosed if the fetal

birth weight was below the 10th percentile for gestational age and gender.

Biological samples

Maternal blood samples were obtained from an antecubital vein into native tubes and centrifuged at room temperature with a relative centrifugal force of 3,000 \times g for 10 min. The aliquots of serum were stored at -20°C until the analyses were performed.

Laboratory methods

Serum Hsp70 levels were measured by using the ELISA Kit of (A HSP70 Bioassay ELISA Kit, U. S. Cat#H1834-37) according to the instructions supplied.

Statistical analysis

The data analyzed by using T-test and least significance differences between control and patient ($P \leq 0.05$) to compare between treatment. For all statistical analyses, $p \leq 0.05$ was considered statistically significant.

Results

Patient characteristics of the study participants are shown in Table (1). There were no statistically significant differences between the two study groups in terms of maternal age and gestational age at blood withdrawal and delivery and the median fetal birth weight was significantly lower in pregnant asthmatics than in healthy pregnant women (3,580 versus 3,230 g, $p \leq 0.05$).

Serum Hsp70 levels

As presented in Table (1), serum Hsp70 levels were significantly higher in pregnant asthmatics than in healthy pregnant women. We determined a cutoff value of Hsp70 concentration (0.30 ng/ml), which can discriminate pregnant asthmatics from healthy pregnant women with 90.0% sensitivity and 85.0% specificity. High Hsp70 level (0.30 ng/ml) was significantly associated with bronchial asthma in pregnancy ($p \leq 0.001$). as compare between Hsp70 serum levels and fetal birth weight in asthmatic women, there were no statistically significant differences ($p \leq 0.05$).

Table (1)**Serum Hsp70 levels and Fetal birth weight of healthy pregnant women and pregnant asthmatics.**

<i>Variable</i>	<i>Controls (n=50)</i>	<i>Asthmatics (n=50)</i>	<i>Statistical sig. (p value)</i>
Age (years)	26 (18-34)	27 (18-36)	NS
Fetal birth weight (gr.)	3,580 (3,450–3,775)	3,230 (2,690–3,550)	<0.05
Serum Hsp70 level (ng/ml)	0.16 (0–0.22)	0.46 (0.38–0.55)	<0.001

Data are presented as median (18–75 percentile) for continuous variables. NS: Not Significant.

Discussion

This study shows a negative correlation between Hsp70 serum level with fetal birth weight in pregnant asthmatics women ($p \leq 0.05$), also the results revealed that an increase in serum Hsp70 levels in asthmatic pregnant asthmatics as compared to maternal and gestational age-matched healthy pregnant women. In healthy pregnant women, serum Hsp70 levels showed a positive correlation with gestational age and an inverse correlation with maternal age (data not show). The latter two observations are in agreement with [4].

The mechanisms that are involved in maintaining a human pregnancy to term, and the switches that lead to a normal labor and pregnancy outcome or indeed an adverse outcome such as miscarriage, preeclampsia, fetal growth restriction or preterm labor, are complex [33]. Reduced fetal birth weight were associated with increased maternal circulating INF- γ and IL-4 positive T cell counts in pregnant asthmatics [25]. According to these data, a connection between asthmatic inflammation and reduced fetal birth weight can be suspected in asthmatic pregnancies, but not with Hsp70 serum levels.

Yang and coworkers reported a significant increase in the rate of detected antibodies against Hsp70 in patients with asthma. In their study, the presence of autoantibodies against Hsp70 was associated with the severity of asthma.

The presence of anti-Hsp70 was also correlated with higher levels of total IgE and IL-4 in asthmatic patients [26]. Elevated serum levels of Hsp70 have recently been demonstrated in patients with chronic obstructive pulmonary disease [27]. However, according to our knowledge, no studies have been performed yet to evaluate the circulating

Hsp70 levels in asthmatic pregnancy in our province. Hsp70 has been suggested to play a role in asthma and lung injury [28,29]. Airway cells (epithelial cells and alveolar macrophages) as well as peripheral blood mononuclear cells showed increased expression of Hsp70 in asthma [30,31]. Since Hsp70 is recognized to have a role in chaperoning antigenic peptides and in facilitating class II peptide assembly, Hsp70 overexpression in professional and nonprofessional antigen presenting cells (APCs) implies a potential role for this protein in antigen processing and/or presentation resulting in an increased activity of APCs, which is essential for the initiation and modulation of the asthmatic immune response in chronic asthma [32]. Furthermore, Hsp70, in Th2 environment, can upregulate CD23 expression of THP-1 cells and alveolar macrophages and, thus, might play an important role in maintaining the chronic bronchial inflammation in asthma [27].

However, the source of circulating Hsps in healthy individuals, as well as in patients with pathological conditions, has not been completely determined yet. It is also not known whether circulating Hsp70 concentration reflects intracellular expression. Furthermore, not only can Hsps be released from viable cells exposed to stressful stimuli by active mechanisms but might also be discharged from necrotic cells in a passive manner. Asthmatic women are at an increased risk for several complications during pregnancy. Schatz and coworkers followed 366 pregnancies of asthmatic women, and in 35% of them, asthma worsened during pregnancy, but in most of the patients, symptoms reverted within 3 months after delivery [24]. The epidemiological observations

indicate that pregnancy may exacerbate asthma. A pregnant woman with asthma is at risk for experiencing preeclampsia, gestational diabetes, placenta praevia, chorioamnionitis, cesarean delivery, and increased maternal hospital length of stay. Adverse perinatal outcomes associated with asthma include preterm delivery, low birth weight, intrauterine growth restriction, perinatal death, and increased infant hospital length of stay [15,16,17].

Several studies have associated elevated Hsp70 levels with higher risk for complications during pregnancy. Among patients who were at risk for preterm delivery, the mean serum concentration of Hsp70 was higher in patients who delivered preterm than in those who delivered at term [9]. High serum concentrations of Hsp70 were also reported in pregnant patients with preeclampsia [8,9,10]. Increased serum Hsp70 levels have recently been found to be associated with systemic inflammation, oxidative stress, and hepatocellular injury in preeclampsia [12]. These data suggest that circulating Hsp70 might play a connecting role in the pathomechanism of asthmatic inflammation and obstetrical/perinatal complications in asthmatic pregnant patients. The fetus is a semi-allograft, since its antigen structure is partly of paternal origin. Several mechanisms have been implicated in the maintenance of immunological tolerance to the embryo/fetus in pregnancy. Pregnancy is characterized by a shift towards a T helper type 2 (Th2) immune response, which maintains pregnancy [34]. Abnormal extracellular Hsp70 levels during pregnancy associated with asthma could initiate proinflammatory immune responses (e.g., proinflammatory cytokine (tumor necrosis factor- α (TNF- α), interleukin (IL)-1 β and IL-6) and chemokine production, conversion of dendritic cells from tolerogenic to immunogenic and stimulation of the cytolytic activity of NK cells and γ/δ T cells), which might lead to pregnancy complications [35,36, 37].

In summary, according to our results, higher circulating Hsp70 levels can be detected in pregnant asthmatic women higher than in healthy pregnant women. Abnormal, asthma dependent Hsp70 levels may play a

role in the altered immunological tolerance in pregnancies of asthmatics, which might result in obstetrical/ perinatal complications. Nevertheless, further studies are needed to determine the role of circulating Hsp70 in the pathogenesis of maternal and perinatal complications of asthma in pregnancy.

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الخلاصة

يعد مرض الربو من الأمراض متعددة الخصائص التي تصيب القنوت التنفسية العليا. وهو من الأمراض المتداخلة حوالي الحمل. ويشكل احد أهم عوامل الخطورة لكلا من إلام والجنين على حد سواء. إن المعلومات المتوافرة عن الربو فيما يخص ميكانيكيته المرضية أثناء فترة الحمل أو حوالي الولادة تكاد تكون نزره. ومنها ما يخص دور بروتين الصدمة الحرارية 70 فالمعروف من الدراسات السابقة إن هذا البروتين ينخفض مستواه أو تركيزه في الحوامل الأصحاء والمفسر باحتمالية التداخل الناعي الضعيف. تهدف الدراسة الحالية إلى تحديد مستوى هذا البروتين في مصلى الحوامل المصابات بالربو مقارنة مع الحوامل الأصحاء وكذلك تقدير وزن الأطفال لديهن. لذلك تم اخذ 50 أمراه حامل مصابة بالربو القسبي مع 50 امرأة حامل غير مصابة بهذا المرض كمجموعة سيطرة (أصحاء). تم ملاحظة زيادة ويفرق معنوي في مستوى وتركيز بروتين الصدمة الحرارية 70 في مصلى النساء الحوامل والمصابات بالربو مقارنة تركيزه في مصلى النساء الحوامل غير المصابات بالربو القسبي. الأمر الذي أوضح بضرورة وجود دراسات مستقبلية لمعرفة دوره المناعي في أثناء فترة الحمل. كما وتبين وجود انخفاض ويفرق معنوي لأوزان الأطفال المواليد لأمهات مصابات بداء الربو مقارنة بالنساء غير المصابات.