Recurrent miscarriages, innate immunity, and autoimmune reaction to chlamydial 60-kDa heat shock protein—is there an association?

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Objective: To evaluate a potential association of immunity to the *Chlamydia trachomatis* 60kDa heat shock protein (ChlamHSP60) and recurrent miscarriages.
Design: Prospective study.
Setting: Outpatient miscarriage clinic of a university-based hospital.
Patient(s): 120 asymptomatic women with a history of recurrent miscarriages.
Intervention(s): None.
Main Outcome Measure(s): Determination of serum immunoglobulin G (IgG) antibodies (Ab) to ChlamHSP60 and human HSP60 and, in parallel, mannose-binding lectin (MBL) and the total hemolytic complement (CH50); medical history and clinical examination, including multiple relevant laboratory determinants.
Result(s): ChlamHSP60 Ab were detected in 24 (20%) of 120 patients. Antibodies to human HSP60 were found in 19 (15.8%) of 120 patients, and more frequently in individuals who tested positive for ChlamHSP60. ChlamHSP60 were statistically significantly associated with antichlamydial IgG Ab. However, antibodies to ChlamHSP60 were not related to medical history, the number of abortions, or the time frame of fetal loss. ChlamHSP60 antibodies were not associated with the relevant variables of the coagulation cascade, a panel of autoimmune parameters including thyroid autoimmunity, deficiencies of the complement system (low MBL), or with antibodies to common infectious diseases. No statistically significant differences were was found when comparing the prevalence of ChlamHSP60 Ab in the study group with recurrent miscarriages and 90 controls (women attending for an annual pelvic examination).
Conclusion(s): Immunity to ChlamHSP60 does not play a major role in the etiology of recurrent miscarriages. (Fertil Steril® 2014;101: 1675–80. ©2014 by American Society for Reproductive Medicine.)
Key Words: Chlamydial 60kDa heat shock protein (Chlam HSP60), *Chlamydia trachomatis*, complement, mannose-binding lectin (MBL), heat shock proteins (HSP), sexually transmitted diseases (STD), recurrent miscarriages, subclinical infection/inflammation markers

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The etiology of recurrent miscarriages is multifaceted. Chromosomal or uterine anomalies, endocrine disorders, coagulation defects, and/or psychological factors have been considered to play an important role, but there is controversy about the significance of subclinical infection/inflammation for early pregnancy loss (1–7). Heat shock proteins (HSP) may play a particular role during the course of early pregnancy, with involvement of HSPs of both human and microbial origin. Heat shock proteins function as molecular chaperons, meditating the folding and unfolding of other proteins, and they are essential for normal protein quality control and homeostasis within the cell. They are key regulators of both the innate and the

Received May 23, 2013; revised February 13, 2014; accepted February 20, 2014; published online March 26, 2014.
W.E.-K. has nothing to disclose. S.S. has nothing to disclose. M.K. has nothing to disclose. T.S. has nothing to disclose.
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Fertility and Sterility® Vol. 101, No. 6, June 2014 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2014.02.048

adaptive immune system and are evolutionary highly conserved (8, 9). The heat shock response is a vital cellular survival mechanism. After exposure to an environmental stressor such as inflammation or toxic chemicals, HSP synthesis is markedly increased, as it is under conditions of rapid cell growth and differentiation. These chaperons are expressed in the endometrium throughout the menstrual cycle and in the decidua during early pregnancy and are important for germ cell differentiation and development (10).

The level of HSP expressed by microbial pathogens is increased at sites of an infection and may exert pathogenic effects on reproductive processes by promoting a persistent inflammatory response via induction of cytokine release (11, 12). *Chlamydia trachomatis* infection, the most common sexually transmitted disease (STD) in industrial countries (13), has a complex immunopathogenesis (14–16). In its persistent form, *C. trachomatis* produces high levels of 60-kDa HSP (HSP60), which is considered a target antigen for autoimmune diseases. Chlamydial HSP60 shares about 50% amino-acid sequence homology with the human HSP60; as a consequence of molecular mimicry, both humoral and cellular autoimmune responses to human HSP60 have been suggested (16–18). Cross-reactivity and immune sensitization to HSPs may cause autoimmune diseases and reproductive failure (19). In in vitro experiments, antibodies to different mammalian HSPs exerted a detrimental effect on mouse embryo development (20).

Little attention has been paid to the role of the innate immune system when evaluating early pregnancy losses. Deficiencies within the complement system such as decreased levels of mannose-binding lectin (MBL) may lead to increased susceptibility and clinical severity of certain microbial infections, inappropriate host defense, and autoimmune reactions (21, 22). Thus, our prospective investigation evaluated the clinical significance of *Chlamydia trachomatis* 60-kDa heat shock protein (Chlam HSP60 Ab) in patients with a history of recurrent miscarriages as well as the potential relationship of immune sensitization to human HSP60, other miscarriage-associated autoimmune factors, and the influence of the innate immune system.

MATERIALS AND METHODS

Participants

The study population consisted of 120 women with a history of at least two spontaneous abortions, who were presenting for the first time at our outpatient miscarriage clinic. Patients with a severe uterine factor abnormality, chromosomal anomalies in either partner, and those for whom it had been <3 months since the previous pregnancy were excluded from the study. None of the women had a history of thrombosis, symptoms of a genital tract infection, or were being treated with antibiotics, corticosteroids, or nonsteroidal antiphlogistic medications. The median age of the women was 37 years (range: 23–44 years).

The median number of miscarriages was three (range: 2–7). In the study group, 98 women had had ≥3 miscarriages (81.7%), 52 had had ≥4 (43.4%), and 24 patients had had ≥5 (20%) repeated spontaneous abortions. For the pregnancy losses, 77.5% had had primary, and 27 women (22.5%) had had secondary losses (i.e., at least one live birth with the same partner). The majority had early spontaneous abortions (<12 week of gestation); 7 women (5.8%) had had late miscarriages only (13–28 weeks of gestation), and 18 women (15%) had a history of both types.

For the control group, we enrolled 90 apparently healthy women who were undergoing an annual pelvic examination in our outpatient clinic. All had a normal obstetric history: no miscarriages, no increased time to pregnancy, no known malignancies, and no symptoms of genital tract infection. All had had a normal course of pregnancy and at least one healthy child. The screen for C-reactive protein (CRP) was negative for all the controls.

The study was approved by the institutional review board (the ethics committee of the university). Informed consent was obtained from all participants.

Analyses

For all participants, a detailed medical history was obtained, and a physical examination was performed. Blood was taken to screen for immunity to chlamydial and human (hum) HSP60. The serum immunoglobulin G antibodies (IgG Ab) to Chlam HSP60 were determined by means of a commercial enzyme-linked immunosorbent assay (cHSP60-IgG-ELISA; Medac), strictly following manufacturer's instructions. Positive and negative controls were assayed in parallel to the test specimens. Positivity was reported as defined; borderline results (±10% of the cutoff) were considered as negative. For further comparison, the results were also expressed as the cutoff index (COI) (optical density of the sample/cutoff).

A comparable assay was used to determine IgG Ab to hum HSP60 (hHSG-IgG-ELISA; Medac) in aliquots of the same serum samples. A positive result was defined according to the assay instructions, and the results were expressed as the COI.

The samples were also used to determine anti-*Chlamydia trachomatis* Ab of the IgG and IgA class with a commercial assay directed to recombinant chlamydial lipopolysaccharide (LPS) fragments on prepared microplates (*Chlamydia trachomatis* IgG-pELISA, *Chlamydia trachomatis* IgA-pELISA; Medac). The tests were performed according to the manufacturer's instructions. Results were expressed as the number and percentage of positive results and as the COI.

Testing was performed by a consistent observer throughout the study. All serum samples were processed blind, without knowledge of the other clinical variables of the patients.

As part of our standard protocol, the following laboratory parameters were determined: full blood count (erythrocytes, leukocytes, platelets, hemoglobin), determinants of the coagulation cascade including activated partial thromboplastin time, fibrinogen, antithrombin (AT) III (activity), protein C, protein S (agglutination test), activated protein C (APC) resistance, factors VIII, XII, XIII (coagulometry), factor V Leiden mutation (polymerase chain reaction [PCR]), homocysteine (high-performance liquid chromatography [HPLC]), MTHFR mutation (PCR), immunologic factors (lupus anticoagulant, anticardiolipin Ab (IgG ELISA), antinuclear Ab (ANA)