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Seropositivity for the human heat shock protein (Hsp)60 accompanying seropositivity for *Chlamydia trachomatis* is less prevalent among tubal ectopic pregnancy cases than individuals with normal reproductive history

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ABSTRACT

Objectives: To investigate the role of anti-human heat shock protein 60 (hHsp60) antibody positivity in the pathogenesis of ectopic pregnancy, following *Chlamydia trachomatis* (CT) infection.
Study design: In a case-control study, serological tests for anti-hHsp60 were performed in ectopic pregnancies (study group) and parturients with normal reproductive histories (control group). All participants in both groups were CT IgG(+). hHsp60 IgG(+) prevalences were compared between the two groups, by semiquantitative ELISA. Data were evaluated using nonparametric and parametric tests and multivariable regression.
Results: After an initial pilot study, two groups were formed: 63 ectopic gestations (study group) and 95 normal parturients (control group), all CT IgG(+). Blood samples from all cases were tested for anti-hHsp60 IgG. Age, gravidity, and practising contraception were higher in the control group, while a history of pelvic infections were more common in the study group. Hsp60 IgG(+) was found to be significantly higher in the control group (63/95, 66.3%) compared to study group (30/63, 47.6%). Regression analysis revealed anti-hHsp60 positivity was an independent factor delineating the two groups.
Conclusion: Immunity to hHsp60 is less common in CT IgG(+) ectopic pregnancies than CT IgG(+) fertile subjects without a history of ectopic pregnancies. Hence, our findings suggest that hHsp60 seropositivity may decrease the probability of an ectopic gestation in subjects with previous CT infections.

Introduction

Chlamydia trachomatis (CT) is the most common cause of pelvic inflammatory disease [1].

Pelvic infection, specifically caused by chlamydia, is known to be associated with tubal factor infertility and tubal ectopic pregnancies. Tubal damage caused by the infection may also be due to cross-immunity, targeting the tubal epithelium. Immunity to heat shock proteins, particularly heat shock protein 60 (Hsp60), is one potential cause of cross-immunological damage to the tubal epithelium [2].

In this study, we investigated the association of anti-Hsp60 antibody positivity in individuals who were seropositive for CT, with tubal ectopic pregnancies.

Materials and methods

This is a case-control study conducted in a state hospital, at the Bağcilar Research and Training Hospital Obstetrics and Gynaecology Department, Istanbul, Turkey between October 2015 and November 2016. Informed consent was obtained from all of the participants, and the study was conducted in accordance with the amendments of the Helsinki Declaration. The protocol was reviewed and approved by the institutional review board (GOKAEK-2014/311), and financial support was provided by the Bagcilar Research and Training Hospital, following evaluation by its Educational Planning Committee (EPK-02/09/2016-08).

A pilot study was performed to estimate the serological prevalence of human Hsp60 IgG positivity, hHsp60 IgG(+), within the CT IgG(+) population. Then, sample sizes needed for the study and the control groups were calculated. Two cohorts, comprising CT IgG(+) ectopic pregnancies (study group) and CT IgG(+) control

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parturients (control group), were formed in planning the study. The study group comprised CT IgG(+) patients, who were diagnosed and treated for tubal ectopic pregnancy in our department and were not transfused before blood sampling. The control group included CT IgG(+) parturients without any history of ectopic pregnancies. The exclusion criteria for both cohorts were having been diagnosed or treated for infertility, recurrent pregnancy losses, a chronic medical or surgical disorder. ELISA tests were performed to define the CT IgG and hHsp60 IgG individuals within the ectopic pregnancy subjects (study group) and normal parturients (control group).

Blood samples were collected from patients, centrifuged at 3000*g* for 10 min and then, stored at −80 °C before evaluation by ELISA. The samples were thawed and then placed, alongside the standards, in microplate wells, which were pre-coated with anti-human monoclonal antibody. hHsp60 IgG (CK-E91542; Eastbiopharm Co. Ltd, HD Biyoteknoloji ve Yasam Bilimleri Ar-Ge Istanbul-Turkey) and CT IgG (CK-E10048; Eastbiopharm, HD Biyoteknoloji ve Yasam Bilimleri Ar-Ge Istanbul-Turkey) were semi-quantified using commercial ELISA kits. Biotin was added to all wells and combined with streptavidin – horseradish peroxidase to form immune complexes. After incubation, the plates were washed to remove any uncombined enzyme. Chromogen solutions A and B were added. Optical densities were determined using an automated ELISA microplate reader (Thermo Scientific Multiskan FC, 2011-06, USA) at 450 nm.

Univariate tests were used to compare dichotomous variables between the two groups. Variables with apparent differences were analysed by a regression model. Statistical analyses were conducted using the SPSS 20.0 and Microsoft Excel software packages. Differences were considered significant at $P < 0.05$.

Results

Initially, we conducted a pilot study to define the prevalence of chlamydia IgG(+) and hHsp60 IgG(+) cases. This pilot study included 90 patients, composed by 60 ectopic pregnancies and 30 control cases, who were normal parturients without any history of ectopic pregnancies, or infertility. We observed that 45% (27/60) were CT IgG (+) in the ectopic pregnancy group and 33.3% (10/30) were CT IgG(+) in the control group ($P = 0.39$). Of the CT IgG(+) subjects, 66% (18/27) were Hsp60 IgG(+) in the ectopic pregnancy group, while 80% (8/10) were Hsp60 IgG(+) in the control group ($P = 0.43$).

In order to compare the Hsp60 IgG(+) prevalences of CT IgG(+) cases between the ectopic pregnancy and control groups, we performed a sample size calculation. For a statistically significant difference of 25% for two cohorts of 80% vs 60%, with a one-to-one enrolment ratio, 81 subjects per group needed to be analysed. Based on the CT IgG(+) prevalences observed in our pilot study, we carried out CT serology studies in an additional ∼70–80 ectopic pregnancies and 90–100 control cases, aiming to obtain two groups of 81 CT IgG(+) subjects, respectively. Hence, we completed performing CT IgG serological screening in a total num ber of 156 ectopic pregnancy and 218 control subjects, yielding positivity rates of 40.4% (63/156) and 43.6% (95/218), respectively ($P = 0.54$) (Fig. 1). These two groups formed the study group (n = 63), who were the CT IgG(+) ectopic pregnancy cases and the control group (n = 95), composed of the CT IgG(+) control subjects. The sera of these patients were then tested for hHsp60 antibody positivity. Consequently, 47.6% (30/63) and 66.3% (63/95) were positive for hHsp60 antibodies in the ectopic pregnancy and the control groups, respectively ($P = 0.02$) (Fig. 2). These two cohorts were compared with respect to their reproductive histories and demographic characteristics (Table 1). Regression analysis revealed that the hHsp60 antibody positivity had an independent association with ectopic pregnancies, not being associated with the

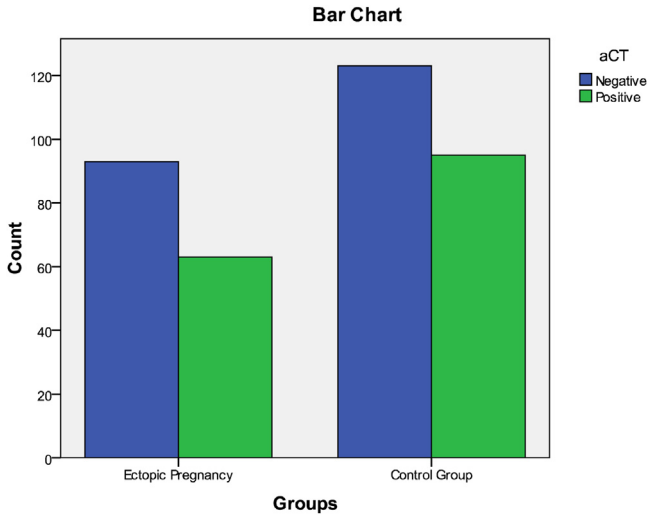

Fig. 1. The proportion of aCT IgG(+) cases in the Ectopic pregnancy and Control groups were similar. *SPSS Version 22*:.

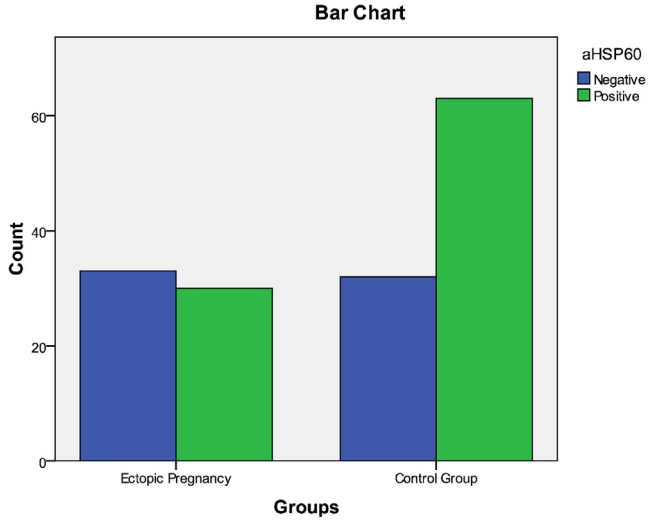

Fig. 2. The proportion of ahHSP60 IgG(+) cases in the Ectopic Pregnancy Group was significantly higher than the Control Group ($P<0.05$). (both groups comprised of aCT IgG(+) patients) *SPSS Version 22*:.

significantly different characteristics between the two groups (i.e., age, gravidity, parity, number of children alive and the proportion of cases conducting a contraceptive method).

Discussion

In this study, we observed that within a CT IgG seropositive sample population, hHsp60 immunity was significantly less prevalent within the tubal ectopic pregnancies group than normal parturients. The patients' ages, gravidity, parity or other reproductive histories and characteristics were similar between the two groups and unrelated to the Hsp60 serological status.

CT seropositivity has been shown to be a marker of tubal damage, causing tubal factor infertility but with high negative predictive values (85–90%), and low positive predictive values (30–65%) [3]. Tubal damage has been proposed to be a major causative agent in the pathogenesis of ectopic pregnancies. We did not note a distinct difference in the chlamydia seropositivity in our pilot study between the ectopic pregnancy and the control groups, in agreement with findings reported by Mouton et al. [4].