Antibodies to the Chlamydial 60 Kilodalton Heat Shock Protein in Women With Tubal Factor Infertility

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ABSTRACT

Introduction: Fallopian tube damage and subsequent infertility are common sequelae of upper genital tract infection with *Chlamydia trachomatis.* This fallopian tube damage is thought to be immune mediated. The 60 kilodalton chlamydial heat shock protein (hsp) may be the key antigen associated with this pathogenic response. Our objective was to study the relationship between antibody response to 60 kilodalton chlamydial hsp and tubal factor infertility (TFI).

Subjects and Methods: Twenty-three women with TFI and 33 women with male factor infertility (controls) were studied. Tubal factor infertility was defined as infertility for one year with hydrosalpinx or distal tubal occlusion. Patients' sera were tested for antibodies to the chlamydial hsp using an enzyme-linked immunosorbent assay (ELISA). A stepwise logistic regression was performed by each patient's age, race/ethnicity, self-reported history of chlamydia infection, gonorrhea, or pelvic inflammatory disease (PID), history of ectopic pregnancy, and antibodies to the chlamydial hsp.

Results: Eighteen of the 23 women with TFI had a positive result on the hsp ELISA (78.6%) versus 23.4% of controls. Risk factors for TFI were a history of PID ($P = 0.022$), "nonwhite" race ($P = 0.004$), history of ectopic pregnancy ($P = 0.027$), and antibodies to the 60 kilodalton chlamydial hsp ($P < 0.001$).

Conclusions: Antibodies to 60 kilodalton chlamydial hsp are strongly associated with TFI. Infect. Dis. Obstet. Gynecol. 6:163–167, 1998. © 1998 Wiley-Liss, Inc.

Chlamydia trachomatis is the most commonly reported bacterial infection in the United States.[1] As a sexually transmitted pathogen, chlamydia can ascend through the female reproductive tract and cause fallopian tube damage. One of the important sequelae of this damage is infertility. Fallopian tube damage is responsible for 36% of cases of female infertility.[2] It is estimated that 75,000 to 225,000 women become infertile every year in the United States as a result of infection. A history of pelvic infection is the most important risk factor for tubal factor infertility (TFI). The likelihood of infertility increases with increased number of episodes of pelvic inflammatory disease (PID), as well as increased severity of these episodes.[3] Despite this link between infections and infertility, clinical

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predictors for TFI are lacking. Most women with TFI do not give a history of prior infection. In one study, women with TFI but without a history of PID were similar to fertile controls in regards to marital status, education, socioeconomic status, and total number of sexual partners.[4]

Recent research has suggested that antibodies to the 60 kilodalton chlamydial heat shock protein (hsp) are associated with TFI. This antigen is a member of the family of "stress" or "heat shock" proteins, a group of highly conserved membrane proteins found in both prokaryotes and eukaryotes. In cell culture models, increased expression of 60 kilodalton chlamydial hsp is associated with arrest of the chlamydial developmental cycle and persistence of infection.[5] Using chlamydial hsp as the antigen in an enzyme-linked immunosorbent assay (ELISA), Toye et al.[6] found a detectable antibody response in 32 of 44 (81%) women with TFI. Likewise, Arno et al.[7] reported that 16 of 21 (76%) women with TFI had antibodies to this protein. It is our hypothesis that this antibody response to 60 kilodalton chlamydial hsp can be correlated to TFI. We report here a study incorporating other demographic variables that are potentially associated with fallopian tube damage.

SUBJECTS AND METHODS

Women attending the Women's Reproductive Center at the University of Kansas Medical Center between 1994 and 1996 were eligible for this study. This facility offers a full range of infertility services, including laboratory services and assisted reproductive technologies. Eligible patients were identified by a review of patient charts and laboratory records. Demographic variables were abstracted at the time of chart review. All women in this study had at least a basic infertility evaluation. This evaluation included a test for tubal patency (hysterosalpingogram and/or laparoscopy), a test of the male partner (semen analysis), and a test for ovulation (basal body temperature chart and/or midluteal progesterone level).

Women were considered to have TFI if they were having regular unprotected intercourse without conception for one year with evidence of distal obstruction, pelvic adhesions, or hydrosalpinx on hysterosalpingogram or laparoscopy. Women were excluded from this group if any other cause of infertility was found, such as endometriosis, abnor-

malities in semen analysis, anovulation, or luteal phase defect. Control patients were selected from the same population. Women were eligible to be controls if they were having regular unprotected intercourse without conception for one year with demonstrated tubal patency on laparoscopy or hysterosalpingogram. Additionally, these women had a diagnosis of male factor infertility as demonstrated by an abnormal semen analysis. Abnormal semen analysis was defined according to World Health Organization criteria.[8] Likewise, women were excluded as controls if any other reason for infertility was identified as stated above. All subjects and controls were tested for infection with *C. trachomatis* with endocervical culture, and no patient in either group was positive.

Antibodies to 60 kilodalton chlamydial hsp were measured by ELISA. Procedures for purifying this protein using an immunoaffinity column have been previously described.[9] Ninety-six well microtiter plates (Immunlon 2, Dynatech, Chantilly, VA) were coated with 100 µL of 1 µg/mL solution of chlamydial hsp in phosphate buffered saline and adsorbed three hours at 37°C. Plates were washed three times with phosphate buffered saline then blocked with 150 µL of 3% bovine serum albumin in phosphate buffered saline per well for 90 minutes at 37°C. One hundred microliters of patients' sera (diluted 1:500 in phosphate buffered saline containing 0.1% bovine serum albumin and 0.2% TWEEN 20) were then added to duplicate wells and incubated for 60 minutes at 37°C. After three additional washes with phosphate buffered saline, 100 µL of a 1:5000 dilution of alkaline phosphatase-conjugated rabbit anti-human IgG (Jackson Immunoresearch Laboratories, West Grove, PA) was then added to each well and incubated for 30 minutes at 37°C. After rinsing three times with phosphate buffered saline containing 0.2% TWEEN, 100 µL of p-nitrophenol phosphate in diethanolamine solution (Sigma, St. Louis, MO) were added to each well and the plates were incubated for 30 minutes at 37°C. Absorbance was then read at 405 nm using an automated plate reader. (ThermoMax, Molecular Devices Corporation, Menlo Park, CA)

Sera from 28 pregnant women were used to define cut-off values as previously described.[6] These pregnant women all tested negative for antibodies to *C. trachomatis* serovar L2 elementary bodies us-

ing a commercially available ELISA (Clark Laboratories, Jamestown, NY). None gave a history of prior infection with *C. trachomatis*, and none had infectious complications with their pregnancies. The mean absorbance of these 28 seronegative samples plus three standard deviations was considered the cut-off value for negative values in the chlamydial 60 kilodalton hsp ELISA. This value was 0.218, and values above this were considered to be positive for antibodies to the chlamydial hsp. Two positive control samples from previously published studies were also used. Tolerance for intra-assay or interassay variance was 10% or less.

The following variables were considered for our multivariate analysis: age greater than 35 years, race/ethnicity (white versus "nonwhite"), a self-reported history of chlamydia infection, a self-reported history of PID, a self-reported history of any infertility-related infection (gonorrhea, chlamydia, or PID), a history of ectopic pregnancy, and results of chlamydial 60 kilodalton hsp ELISA. An initial analysis was done with chi-square tests and variables found to be associated with TFI were used in a stepwise logistic regression. Odds ratios and *P* values were calculated using the permutation-based logistic regression algorithms of LogXact-Turbo version 1.3 (Cambridge, MA). The human subjects committee of the University of Kansas Medical Center approved this protocol.

RESULTS

Twenty-three women with TFI as the sole cause of their infertility were identified. Likewise, 33 women with male factor infertility were identified as controls. Eighteen of the 23 women with TFI (78.6%) were positive for antibodies to the chlamydial hsp. Selected variables for subjects and controls are shown in Table 1.

Results of the stepwise logistic regression are shown in Table 2. Age and a self-reported history of infertility-related infection were not significant in the models that were considered. As shown in the table, the following variables were significantly associated with TFI: history of PID (adjusted odds ratio 28.8, *P* = 0.022), history of ectopic pregnancy (adjusted odds ratio 16.8, *P* = 0.027), nonwhite race (adjusted odds ratio 57, *P* = 0.004), and antibodies to 60 kilodalton chlamydial hsp (adjusted odds ratio 30.6, *P* < 0.001).

TABLE I. Selected demographic variables for subjects and controls[a]

Variable	Subjects (n = 23)	Controls (n = 33)	P value
History of ectopic pregnancy	8/23 (34.8%)	1/33 (3.0%)	0.002
History of PID	6/23 (26.1%)	0/33 (0%)	0.003
History of chlamydia infection	3/23 (13.0%)	1/33 (3.0%)	NS[b]
History of PID, gonorrhea, or chlamydia	9/23 (39.1%)	1/33 (3.0%)	<0.001
Nonwhite race	7/23 (30.4%)	0/33 (0%)	0.001
Age >35 y	8/23 (34.8%)	8/33 (24.3%)	NS
Positive for antibodies to the chlamydial heat shock protein	18/23 (78.3%)	8/33 (24.3%)	<0.001

[a]Significance was calculated by chi-square test.
[b]NS, not significant.

CONCLUSIONS

Antibodies to chlamydial 60 kilodalton hsp are strongly associated with TFI. As reported in two previous studies, a large majority of women with TFI have antibodies to this antigen. The difference between this study and previous studies is that we carefully defined a control group and examined confounding factors associated with TFI. Most serological studies involving human responses to chlamydia have not included well-defined control groups.[10] The association between hsp 60 antibodies and TFI remained when other confounding variables were included in a multivariate analysis. It is the only risk factor present in a majority of women with TFI.

Other factors associated with TFI are consistent with previous studies. A history of PID was also a predictor of TFI in this study. As stated previously, this is the risk factor most consistently associated with TFI.[3] However, a majority of women with TFI in this study (17 of 23 women, or 74%) did not report PID. In a larger multicenter trial, 84% of women with TFI do not give a history of PID.[4] Also similar to previous studies, nonwhite race was associated with TFI. This is likely due to increased rates of sexually transmitted diseases among minorities.[3] Ectopic pregnancy due to fallopian tube damage presumably shares a common pathogenesis with TFI, hence the finding that a history of ectopic pregnancy predicts an increased risk of TFI. In one large multicenter case-control study from France, predictors of ectopic pregnancy include a

TABLE 2. Results of multivariate analysis[a]

Variable	Unadjusted odds ratio	Adjusted odds ratio	95% confidence interval	Significance
Hsp 60 Antibodies	10.6	30.6	4.3 to infinity	0.0001
Nonwhite race	18.6	57	3.8 to infinity	0.0037
History of ectopic pregnancy	16.2	16.8	1.36 to infinity	0.027
History of PID	14.6	28.8	1.59 to infinity	0.022

[a]Significance was calculated by permutation based, stepwise logistic regression algorithm.

history of symptomatic sexually transmitted disease, history of PID, serological evidence of prior infection with chlamydia, and a recent partner with urethritis.[11] Unlike previous studies, age was not associated with an increased risk of TFI. Also unlike previous studies, a history of sexually transmitted diseases was not associated with TFI. Grodstein et al.[12] showed that women with TFI more commonly report a history of gonorrhea. Along with chlamydia, gonorrhea is known to be an upper genital tract pathogen capable of causing tubal damage and infertility. This lack of an association between TFI and a self-reported history of gonorrhea might reflect the national trend in the prevalence of gonorrhea: the number of cases of gonorrhea in the United States has steadily decreased since 1975.[13] There were 266,507 cases of gonorrhea in women reported to the Centers for Disease Control and Prevention in 1991, and 188,650 were reported in 1995, a 29% decrease. Only one patient in the study reported a history of gonorrhea, and she was in the subject group.

This study does not address the pathogenic mechanisms by which an immune response to 60 kilodalton chlamydial hsp causes TFI. This is an active area of research and at least three theories have been put forward. Morrison et al.[14] explained the pathogenic response to chlamydia based on differences between the Th1 and Th2 lymphocyte subsets. According to this hypothesis, protective responses are driven by a Th2 response. Th2 lymphocytes would stimulate the production of antibodies, and these antibodies would provide a localized mucosal defense against attachment of the chlamydial elementary body to the tubal epithelium. Th1 cells stimulate macrophages and cell-meditated immunity. If the hsp is the antigenic stimulus for a Th1 response, activated macrophages and pro-inflammatory cytokines could lead to immune-mediated fallopian tube damage. Witkin[15] has proposed a theory based on the homology between human and chlamydial hsp. Heat shock proteins are highly conserved across species. According to this hypothesis, sensitized lymphocytes develop to hsp 60. These lymphocytes would then cross react with shared epitopes of the human hsp and lead to autoimmune destruction of the fallopian tube. Recently, Kimani et al.[16] reported that antibodies to chlamydial 60 kilodalton hsp are a risk factor for the development of PID due to *C. trachomatis*. Based on their analysis of HLA types, they suggest that CD8 cytotoxic lymphocytes specific for chlamydial hsp lead to damage. These lymphocytes would lyse infected fallopian tube epithelial cells as the pathogenic mechanism.

In summary, antibodies to the 60 kilodalton chlamydial hsp are strongly associated with TFI in this study from a single institution. Further research should concentrate on the role this antigen plays in the immunopathogenesis of fallopian tube damage. Additionally, testing for this antibody response may be a clinically useful test for the evaluation of the infertile woman.

ACKNOWLEDGMENTS

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