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Serologic Evidence of Past Infection with *Chlamydia trachomatis*, in Relation to Ovarian Cancer

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Pelvic inflammatory disease has been inconsistently linked with ovarian cancer. We measured antibodies to *Chlamydia trachomatis,* to chlamydial heat shock protein (CHSP) 60, and to CHSP10, in 117 women with ovarian cancer and in 171 age- and ethnicity-matched population-based control subjects from Oahu, Hawaii. IgG antibodies to serovar D of chlamydia elementary bodies (EB) and IgG antibodies to CHSP60-1, CHSP60-2, CHSP60-3, and CHSP10 were detected using an ELISA assay. The probability of having ovarian cancer was 90% greater in women with the highest, compared with the lowest (optical density, ⩾0.40 vs. <0.10), levels of chlamydia-EB antibodies ($P = .05$). There was also a monotonic trend ($P = .09$) in ovarian cancer risk associated with CHSP60-1 but not with CHSP60-2, CHSP60-3, or CHSP10. These data suggest that past or chronic persistent infection with chlamydia may be a risk factor for ovarian cancer.

Ovarian cancer is an often-fatal disease for which prevention strategies have been limited, in part, by a lack of understanding of the underlying biology. In a previous report, we have suggested that pelvic inflammation may play a role in the development of ovarian cancer [1]. Inflammation involves DNA damage and repair, oxidative stress, and elevation of cytokines and prostaglandins—all of which are mutagenic. A cause of profound chronic pelvic inflammation is pelvic inflammatory disease (PID) [2]. PID has been linked to ovarian-cancer risk in some [3,4] but not in all [5] studies.

One-quarter to three-quarters of proven cases of PID are caused by either *Neisseria gonorrhoeae* or *Chlamydia trachomatis* ascending into the upper genital tract, where they inflame the endometrium, fallopian tubes, and ovarian epithelium [6]. Of the 2 pathogens, *C. trachomatis* is the more common pathogen in American women [2]. The chronic inflammation caused by repeated or persistent *C. trachomatis* infection may occur because the chlamydial outer-membrane 60-kDa heat-shock protein (CHSP60) and, possibly, the chlamydial outer-membrane 10-kDa heat-shock protein (CHSP10), cross-react with the human cellular 60-kDa heat-shock protein (HHSP60) and the human cellular 10-kDa heat-shock protein (HHSP10) and initiate auto-antibodies to self.

We used pilot data from a population-based case-control study to assess whether IgG antibodies to chlamydia, CHSP60, and CHSP10 were associated with an elevated risk of ovarian cancer.

Subjects, materials, and methods. Subjects for this serologic analysis were part of a population-based case-control study that was conducted in Hawaii [7]. Case subjects were residents of Oahu who were diagnosed, between 1 July 1993 and 30 June 1999, in any of the major hospital centers on Oahu, with histologically confirmed, primary, epithelial ovarian cancer. Eligible women were 18–84 years old. Of the 291 eligible women, 218 (75%) were interviewed.

Control subjects were female residents of Oahu who were interviewed by the Health Surveillance Program of the Hawaii Department of Health, in an annual survey of a 2% representative sample of all of the households in the state (survey refusal rate, <5%). Women who were ⩾65 years old were selected from Health Care Financing Administration participants. Potential control subjects were selected from the insurance pool so that the ethnic and 5-year–age-group distribution would match that of the case group, with a 1:1 ratio. Additional eligibility criteria for control subjects included the requirements that they have at least one intact ovary and that they had no prior history of ovarian cancer. Of the 416 eligible women, 284 (68%) were interviewed.

Women were interviewed in their homes by trained interviewers. On average, case subjects were interviewed <4 months after being diagnosed. The questionnaire requested data on reproductive and gynecological history, contraceptive history,

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medical history, and family history and information on lifestyle practices.

We were able to collect blood samples from 146 (67%) of the interviewed case subjects and from 192 (68%) of the interviewed control subjects. Blood samples were processed, within 1–2 h after being collected, by a laboratory technician. For our analysis, we selected 117 case subjects and 171 control subjects, for whom we had complete questionnaires and tumor-registry (e.g., histology) information and adequate blood samples.

Serologic testing for IgG antibodies to serovar D of *C. trachomatis* elementary bodies (EB), the extracellular form of the chlamydia bacteria, as well as serologic testing for IgG antibodies to CHSP60-1, CHSP60-2, CHSP60-3, and CHSP10, was conducted in the reference laboratory of one of the authors (R.C.B.), by an ELISA technique. The CHSP genes were cloned from serovar D genomic DNA into pET Vector (NOVagen). The histidine-tagged recombinant proteins were expressed in *Escherichia coli* BL 21 and were purified using an Ni-NTA agarose column (QIAGEN). Flat-bottomed (96-well) polystyrene microtiter plates (Corning Science Products) were coated with 100 μL of recombinant CHSP60-1, -2, and -3 (0.1 μg/well) diluted in 0.1 *M* carbonate buffer (pH 9.6), in each well. Plates were incubated overnight at 4°C and then were washed 3 times with PBS with 0.05% Tween 20. Nonspecific binding sites were blocked by addition of 150 μL of 3% bovine serum albumin (BSA) in PBS to each well, for 2 h at 37°C. After 2 washings with PBS with Tween 20, 100 μL of human serum diluted to 1:250 in PBS with 0.5% BSA were added, in duplicate, and plates were incubated overnight at 4°C. After 4 washings with PBS with Tween 20, 100 μL of 1:2000-diluted peroxidase-conjugated–antihuman IgG secondary antibody were added, and plates were incubated for 2 h at 37°C. After 4 washings with PBS with Tween 20, 100μL of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) substrate was added and was allowed to develop for 5 min–2 h, at room temperature in the dark, until the positive control wells on each plate reached the optical density (OD) value of 1.0, at OD_{405}, on a microplate reader. Final readings are based on the mean of duplicate runs. All assays were conducted by personnel who were unaware of case/control status. The intraassay coefficients of variation were 0.06 for chlamydia antibodies and 0.09 for CHSP60-1; both are excellent representations of intraassay replication. Among masked replicates sent to the laboratory, the interassay coefficients of variation were 0.68 for chlamydia and 0.36 for CHSP60-1; both are representations of moderate interassay variability.

Each of the antibody levels tested was measured in OD units (range, 0–0.4 or greater). Because the distributions of antibody OD units were highly skewed in controls, we log-transformed all OD units when considering them as continuous measures, and we categorized OD units into neat, whole-number categories when considering them as discrete measures. Odds ratios (ORs), with corresponding 95% confidence intervals (CIs), were calculated, as the primary measure of the size of the effect. Because matching was based on frequencies for only 2 broad criteria—age within 5-year intervals and ethnicity—we did not preserve the "match" in the analyses. ORs were adjusted for any residual effect of age, family history of ovarian cancer in any first-degree relative, tubal ligation, nulliparity versus any parity, and years (continuous) of use of oral contraception, in unconditional logistic-regression models.

Results. Women in the study were divided into the following age groups: ⩽44 years old, 45–54 years old, 55–64 years old, and ⩾65 years old; approximately half of the women were Asian, and one-third were white non-Hispanic; most had received some education beyond high school (table 1). Few women reported having had PID.

The heat-shock protein most strongly and most significantly correlated with chlamydia-EB IgG was CHSP60-1 (Pearson correlation coefficient, 0.44; $P < .0001$). The correlations between chlamydia EB and CHSP60-2, between chlamydia EB and CHSP60-3, and between chlamydia EB and CHSP-10 were lower (Pearson correlation coefficients, 0.23, 0.08, and 0.13, respectively), and, of these, only the correlation between chlamydia EB and CHSP60-2 was statistically significant ($P = .002$). Among control subjects, we found no significant associations between any of the risk factors presented in table 1, such as age, and log chlamydia EB or log CHSP60-1, CHSP60-2, CHSP60-3, and CHSP10 antibodies, with the exception that chlamydia heat-shock–protein OD units were lower in white women than in women of other ethnic groups.

We next examined the associations between quartiles of OD units for chlamydia EB, CHSP60-1, CHSP60-2, CHSP60-3, and CHSP10, in women with ovarian cancer and in women without ovarian cancer (table 2). After adjustment for possible confounding factors, we found that women with ovarian cancer (case subjects) were more likely than women without ovarian cancer (control subjects) to have high levels of chlamydia-EB OD units (top 25th percentile, ⩾0.4; $P = .05$). The probability of having ovarian cancer was 90% greater among women with the highest levels of chlamydia OD units, compared with the lowest levels. There was also a monotonic, although nonsignificant ($P = .09$), trend in ovarian-cancer risk associated with the increase of OD units of CHSP60-1. There was no trend toward a higher risk of ovarian cancer among women with higher CHSP60-2, CHSP60-3, or CHSP10 serologies.

We also considered log chlamydia-EB OD units and log CHSP OD units, as continuous measures. After adjustment for covariates, we found that log chlamydia-EB antibodies (OR, 1.3; 95% CI, 1.0–1.6)—but none of the heat-shock proteins—were significantly associated with ovarian-cancer risk.

Discussion. We found that women with ovarian cancer

Table 1. Frequencies of demographic and reproductive characteristics, by case/control status.

Variable	Case subjects, no. (%)	Control subjects, no. (%)	χ^2 (*P*)
Age group, years			
⩽44	32 (27.4)	35 (20.5)	2.56 (.464)
45–54	32 (27.4)	59 (34.5)	
55–64	20 (17.1)	30 (17.5)	
⩾65	33 (28.2)	47 (27.5)	
Ethnic group			
White	32 (29.1)	56 (33.5)	2.47 (.291)
Asian	52 (47.3)	84 (50.3)	
Other	26 (23.6)	27 (16.2)	
Education			
Less than high school	32 (13.7)	13 (7.6)	4.47 (.107)
High school	52 (22.2)	30 (17.5)	
Post high school	26 (64.1)	128 (74.9)	
Family history of ovarian cancer			
No	111 (94.9)	169 (98.8)	4.03 (.045)
Yes	6 (5.1)	2 (1.2)	
Live births, no.			
0	25 (21.4)	18 (10.5)	6.43 (.001)
⩾1	92 (78.6)	153 (89.5)	
Tubal ligation			
No	103 (88.0)	124 (72.5)	10.0 (.002)
Yes	14 (12.0)	47 (27.5)	
Oral contraception, years			
0	79 (67.5)	75 (43.9)	18.7 (0)
<1–4	21 (17.9)	47 (27.5)	
5–9	14 (12.0)	28 (16.4)	
⩾10	3 (2.6)	21 (12.3)	
Menopausal status			
Premenopausal	46 (41.1)	63 (41.2)	0 (.99)
Postmenopausal	66 (58.9)	90 (58.8)	
Self-reported PID			
No	114 (97.4)	171 (100.0)	4.43 (.04)
Yes	3 (2.6)		
Self-reported gonococcalor chlamydial cervicitis			
No	111 (94.9)	165 (96.5)	0.46 (.50)
Yes	6 (5.1)	6 (3.5)	

are more likely to have high levels of IgG antibodies to *C. trachomatis* serovar-D EBs. Although it is not a significant result, antibodies to CHSP60-1 are also higher in individuals with ovarian cancer. Our data are consistent with some, but not all studies, that evaluate the link between ovarian cancer and PID [3,4]. Risch and Howe [4] have reported that women with ovarian cancer are 50% more likely than women without ovarian cancer to report that they had ⩾1 episode of PID, after adjustment for other risk factors. The risk was more pronounced for women who reported recurrent episodes of PID and for women who reported fertility problems; both are groups in which chronic pelvic inflammation would be expected. Studies that do not show an association between self-reported PID and ovarian cancer [5,6] involved few women who reported previous PID: 2% of controls, compared with 11% of US women, in a population survey [8]. The low frequency of self-reported PID, a socially stigmatized and underdiagnosed condition, may well reflect misclassification, which may result in underestimation of the true association between PID and ovarian cancer.

Table 2. Frequencies and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) for chlamydia elementary bodies and chlamydial heat-shock protein (CHSP)–antibody optical-density (OD) units, in case and control subjects.

	Chlamydia EB[a]			CHSP60-1[b]			CHSP60-2[c]			CHSP60-3[c]			CHSP10[c]		
OD units	Case subjects	Control subjects	OR (95% CI)	Case subjects	Control subjects	OR (95% CI)	Case subjects	Control subjects	OR (95% CI)	Case subjects	Control subjects	OR (95% CI)	Case subjects	Control subjects	OR (95% CI)
<0.10	37	59	1.0	73	117	1.0	101	142	1.0	54	86	1.0	51	59	1.0
0.10–0.199	26	50	0.7 (0.4–1.5)	20	32	1.0 (0.5–2.1)	11	22	0.6 (0.2–1.3)	38	56	1.1 (0.6–2.0)	36	62	0.7 (0.4–1.3)
0.20–0.399	22	35	1.2 (0.6–2.6)	13	12	1.3 (0.5–3.3)	5	7	1.2 (0.3–4.6)	19	20	1.4 (0.7–3.1)	21	41	0.7 (0.3–1.4)
⩾0.40	32	27	1.9 (0.9–3.9)	11	9	1.6 (0.6–4.2)				6	8	1.2 (0.4–3.9)	9	8	1.0 (0.3–3.2)

NOTE. ORs were adjusted for age, family history of ovarian cancer, tubal ligation, nulliparity/any parity, and years (continuous) of oral-contraceptive use. CHSP, chlamydial heat shock protein; CI, confidence interval; EB, elementary bodies; OD, optical density.

[a] Test for trend, $P = .05$

[b] Test for trend, $P = .09$

[c] Test for trend, $P > .10$

PID that results from *C. trachomatis* infection often leads to tubal infertility. In a landmark prospective study, 15% of women who had had 1 episode of PID became infertile, and this figure increased to 50% in women who had had ⩾3 episodes [9]. Chlamydia IgG antibodies were detectable in 60%–90% of women with tubal infertility, in 30%–50% of infertile women without tubal occlusion, and in 0–35% of pregnant women or blood donors [10]. The pathophysiology by which tubal infertility occurs is chronic tubal inflammation. Chlamydial PID also causes periadnexal inflammation, scarring, and adhesions, thus involving the epithelial lining of the ovaries.

Up to one-quarter of women who have had PID suffer recurrences [9]. It is unclear whether these recurrences represent acquisition of a new infection, chronic persistent infection, or an autoimmune phenomenon triggered by the initial infection. In monkeys, chronic persistent infection with *C. trachomatis* in the fallopian tubes leads to a strong, long-lasting CHSP60 response [11]. Furthermore, a high level of CHSP60 in response to experimental chlamydia infection is correlated with more-severe inflammatory pathology. In women, antibodies to CHSP60 were found in 6%–25% of fertile women with a serology positive for chlamydia, in 48%–60% of women with chlamydial PID, and in 80%–90% of women with chlamydia-associated tubal infertility [2]. Female sex workers who were prospectively evaluated for incident *C. trachomatis* were 2–3 times more likely to develop PID if they had CHSP60 [12]. Antibodies to HHSP60 and to CHSP60 share 48% of amino acid sequences [13]. These observations suggest (1) that recurrent and/or chronic persistent *C. trachomatis* upper-genital-tract infection induces immunity to CHSP60 and that (2) CHSP60 is a marker of chronic pelvic inflammation.

CHSP10 is an antigen found on the surface of *C. trachomatis.* In a limited number of studies, it too has been linked with tubal infertility, although, in infertile women, the rates of seropositivity for CHSP10 are lower than those for CHSP60 [14]. Furthermore, as we have found in the present study, the correlation between CHSP10 antibodies and CHSP60 antibodies is poor, poorer even than the correlation between CHSP60 and chlamydia-EB antibodies [15].

Strengths of this study include the population-based selection of case and control subjects; the standardized collection and storage of blood samples; the measurement of chlamydia-EB and CHSP antibodies at a reference/research laboratory, with laboratory personnel who were unaware of case-control status; and the evidence of an independent effect, after adjustment for potentially confounding or mediating factors (such as nulliparity and oral-contraceptive use). Limitations of this study include the small number of subjects and the low participation in the blood-ample collection (146/291 [50%] of all eligible case subjects; 192/435 [44%] of all eligible control subjects). Low participation rates may have biased our findings, if exposure to chlamydial infection is a determinant of participation. Pregnancy-associated ORs and oral-contraceptive use, as well as other confounders, were similar for subjects who were involved only in the interview and for subjects who also participated in the blood-sample collection. This gives us some additional confidence that our results are not biased. Still, concerns about potential bias cannot be entirely eliminated. Furthermore, although the intraassay coefficient of variation for the assays of interest was low, the interassay coefficient of variation was substantial. Such misclassification would generally reduce the observed strength of the association. In summary, our findings suggest that past or chronic persistent chlamydia infection (the most common cause of PID) and CHSP60-1 (a marker of chronic upper-genital-tract inflammation) may be risk factors for ovarian cancer.

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