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Chlamydial Pgp3 seropositivity and population attributable fraction among women with tubal factor infertility

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Short summary

Chlamydia trachomatis Pgp3 seropositivity is associated with tubal factor infertility (TFI) in non-black women without endometriosis. Past chlamydial infection may account for TFI in 20% of these women.

Abstract

Background

Chlamydial infection is associated with tubal factor infertility (TFI); however, assessment of prior chlamydial infection and TFI is imperfect. We previously evaluated a combination of serological assays for association with TFI. We now describe the chlamydial contribution to TFI using a newer *Chlamydia trachomatis* Pgp3 enhanced serological (Pgp3) assay.

Methods

In our case-control study of women 19–42 years old with hysterosalpingogram-diagnosed TFI (cases) and non-TFI (controls) in two U.S. infertility clinics, we assessed possible associations and effect modifiers between Pgp3 seropositivity and TFI using adjusted odds ratios (aOR) with 95% confidence intervals (CI) stratified by race. We then estimated the adjusted chlamydia population attributable fraction (aPAF) with 95% CI of TFI.

Results

All black (n=107) and 618 of 620 non-black women had Pgp3 results. Pgp3 seropositivity was 25.9% (19.3–33.8%) for non-black cases, 15.2% (12.3–18.7%) for non-black controls, 66.0%

(95% CI 51.7–77.8%) for black cases, and 71.7% (59.2–81.5%) for black controls. Among 476 non-black women without endometriosis (n=476), Pgp3 was associated with TFI (aOR 2.6 [1.5–4.4]), adjusting for clinic, age, and income; chlamydia TFI aPAF was 19.8% (95% CI 7.7–32.2%) in these women. Pgp3 positivity was not associated with TFI among non-black women with endometriosis nor among black women (regardless of endometriosis).

Conclusions

Among non-black infertile women without endometriosis in these clinics, 20% of TFI was attributed to chlamydia. Better biomarkers are needed to estimate chlamydia TFI PAF, especially in black women.

Introduction

Untreated genital *Chlamydia trachomatis* infection ("chlamydia") may ascend from the lower genitourinary tract to the upper genitourinary tract, leading to pelvic inflammatory disease (PID)(1). The subsequent inflammation can lead to scarring, fallopian tube obstruction, and tubal factor infertility (TFI), with repeated PID episodes and more severe PID increasing TFI risk(2). US women of black race have higher rates of reported chlamydial infections than women of other races(3); however, little is known about ascending chlamydial infection that may ultimately result in chlamydia-associated TFI in women of different racial groups.

Chlamydia serological assays can identify women who have been infected with chlamydia. Seropositivity with these assays has been associated with reproductive chlamydial complications, including TFI(4, 5). Consequently, these assays have been used to estimate TFI population attributable fraction (PAF) related to prior chlamydia(6, 7), although rarely stratified by race. Unfortunately, to date most serological assays have poor sensitivity for measuring prior chlamydia in women(8) regardless of race, resulting in estimates that may not reflect actual chlamydia-related TFI. We previously investigated a relationship between different chlamydia serological assays and hysterosalpingogram (HSG)-diagnosed TFI stratified by race using a reference standard of positivity by the Medac IgG plus assay or a research elementary body ELISA assay; however, we did not find an independent association between chlamydia seropositivity and TFI in black women nor in non-black women(9).

C. trachomatis Pgp3 (plasmid gene product 3) serological assays have significantly higher sensitivity (74–83%) (10-12) than commercially available major outer membrane protein

(MOMP) peptide-based commercial assays (46–60%) for detecting serum IgG antibodies to *C. trachomatis*. The specificity of Pgp3 assays is comparable to other MOMP peptide-based serological assays(11, 12). Additionally, Pgp3 antigen has been associated with upper tract infection in mice, suggesting possible utility of Pgp3 serology for upper tract disease in humans(13).

Both the Pgp3 indirect ELISA and Pgp3 double antigen (enhanced) ELISA assays have been used to evaluate for prior chlamydia using nationally representative data from the United Kingdom(14); however the enhanced assay demonstrates longer serum antibody detection than the indirect assay(8, 12, 15). In this analysis, we used data from our original case-control study which assessed chlamydia seropositivity in women with and without TFI, to determine whether chlamydia seropositivity measured with the enhanced Pgp3 assay is associated with TFI. We also estimated the chlamydia TFI PAF among women with infertility, and examined the data stratified by race to determine the proportion of TFI that might be averted with chlamydia prevention and prompt diagnosis and treatment.

Materials and Methods

Design

Using data from our previous case-control study of women with TFI and other non-TFI infertility(9), we assessed Pgp3 seropositivity stratified by black and non-black race, and evaluated for an association of Pgp3 seropositivity with TFI. The methods for the initial study are described in detail in the original publication(9). In brief, women 19–42 years of age were

recruited from two infertility clinics in Birmingham, Alabama, and Pittsburgh, Pennsylvania from October 2012 through June 2015. Women with infertility (defined as lack of intrauterine pregnancy within a 12-month period despite regular intercourse and lack of contraception) were eligible for inclusion if they had an HSG within 1 year of enrollment.

Definitions

Similar to our previous analysis, women with infertility with evidence of unilateral or bilateral fallopian tube blockage on HSG (lack of free dye spill into the pelvic cavity) were defined as having HSG-diagnosed TFI. Controls were women with non-TFI infertility (enrollment HSG showed bilateral patent fallopian tubes) with no history of prior surgery to repair tubal blockage and no prior ectopic pregnancy. Cases were matched to controls by race, with one control per case for black women, and three controls per case for non-black women powered based on the relationship between chlamydia prevalence and TFI. Data on past medical history, including previously reported sexually transmitted infections and PID were obtained from medical record abstraction and/or patient interview. Women with self-reported or medical record documentation of endometriosis during a surgical procedure were categorized as having a history of endometriosis.

Informed consent was collected per the primary study and ethical review was performed by the institutional review boards (IRBs) of the University of Alabama at Birmingham and the University of Pittsburgh. Centers for Disease Control and Prevention (CDC) review determined that CDC was not engaged in human subjects' research as CDC investigators were not primarily

involved in data collection and therefore CDC IRB review was not required. IRB review was also not required at the University of Bristol.

Data collection and laboratory methods

After informed consent was collected, women were enrolled, interviewed, and their clinical records were reviewed. Sera were collected for chlamydia serology and stored at CDC. The enhanced Pgp3 IgG ELISA assay was used to measure *C. trachomatis* antibody on sera shipped to a collaborator's laboratory at Imperial College London following previously published methods(12).

Analytic methods

We compared categorical characteristics of women with and without TFI by HSG using chi square, Cochrane-Mantel-Haenzel or Fisher's exact tests, and continuous characteristics using the Wilcoxon rank sum test. P values of less than 0.05 were considered to be statistically significant. Pgp3 seropositivity was determined by race overall and by sub-category for cases and controls with 95% CI (Wald or Wilson CIs were used as appropriate). We used multivariable logistic regression models to evaluate the primary relationship (using odds ratios [OR]) of Pgp3 seropositivity with TFI. Our models were constructed to adjust for a priori potential confounders (woman's age, clinic location, and household income); identified potential confounders of the relationship between Pgp3 seropositivity and TFI; and identified effect modifiers. Potential confounders were evaluated by the z test, and effect modifiers were evaluated by the deviance test. The final multivariable model was stratified by the presence or absence of endometriosis, the identified effect modifier. We calculated the unadjusted PAF

using logistic regression to describe the relationship between Pgp3 and TFI and used our same adjusted model for the relationship between Pgp3 and TFI to determine the adjusted PAF(16). Statistical analyses were completed using SAS 9.4 and R 3.6.3.

Results

Characteristics of cases, controls, and chlamydia seropositivity

Similar to our first analysis(9), we examined a population of 784 enrolled women, of whom 727 women were included as TFI cases or controls by HSG. Of these 727 women, the enhanced Pgp3 serology was successfully performed in 725: 186 cases and 539 controls. Women included in the overall analysis (n=725) had a median age of 32.0 years (IQR 29.0–35.0). A majority of the enrolled women with Pgp3 serology data came from the Pittsburgh clinic (n=455, 62.8%) and had an annual household income of greater than or equal to $75,000 (n=407, 61.3%). Educational attainment was similar comparing cases and controls.

When stratified by race, the 47 black case women had statistically significantly higher prior non-marijuana drug use, more infrequent history of hormonal contraception use, and a higher proportion of chronic pelvic pain than the 60 black control women (Table 1). Non-black case women (n=139) had statistically significantly lower household incomes, and more often had trichomoniasis (p=0.01); endometriosis (p<0.01); prior abdominal or pelvic surgery (p=0.04), and were more often chlamydia seropositive (p<0.01) than the 479 non-black control women. The proportions with prior PID did not differ between black case and control women, nor non-black case and control women (Table 1).

Among all women, Pgp3 seropositivity was higher among cases (36.0%) than controls (21.5%, p<0.001). Pgp3 seropositivity was also higher among non-black case women (25.9%) compared to controls (15.2%, p<0.01) (Table 1 and Figure 1). Among black women, however, Pgp3 chlamydia seropositivity was non-statistically significantly lower in cases (66.0%) than controls (71.7%) (p=0.5) (Table 1). A history of chlamydia was non-significantly higher in black case women than controls.

Of all women with a history of chlamydia, Pgp3 seropositivity was 69.9% (79.4% among black women and 61.5% among non-black women). Among black women with no history of chlamydia, 64.4% of women were Pgp3 seropositive.

Among the 28 women with a history of PID (in whom we might expect higher chlamydial seropositivity than the general study population), we observed a statistically significantly higher seropositivity among TFI cases (85.7% [95% CI 60.1–96.0%]) than controls (28.6% [11.7–54.6%]). We also found higher chlamydia seropositivities among black and non-black case women with a history of PID and among those with a history of chlamydia compared to control women in the same racial category as the cases, although the differences were not statistically significant (Figure 1).

Association of Pgp3 seropositivity with TFI

Pgp3 seropositivity was associated with TFI among all non-black women (OR 1.9 [95% CI 1.2–3.1]) (Table 2) but not among black women (OR 0.8 [95% CI 0.3–1.8]). Additionally among non-black women with prior chlamydia, the magnitude of the OR for Pgp3 and TFI was non-significantly higher than the OR among non-black women overall, with a trend toward statistical

significance and large confidence interval (4.6 [91% CI 1.0–34.1]). The OR for Pgp3 and TFI among women with prior PID was 32.0 with a very large confidence interval (95% CI 2.3–1341.4). There was not a statistically significant association of seropositivity with TFI among non-black women with a history of both PID and chlamydia (OR 3.0 [95% CI 0.1–170.8]). We also did not find statistically significant associations between Pgp3 seropositivity and TFI among black women, either overall or among those with prior PID nor prior chlamydia (Table 2).

In our multivariable analysis, the presence of endometriosis modified the independent association of Pgp3 seropositivity with TFI for non-black women. We found a significant association between Pgp3 seropositivity and TFI among non-black women without endometriosis (aOR 2.6 [95% CI 1.5–4.4)] but not among non-black women with endometriosis (aOR 0.4 [95% CI 0.1–1.3]) (Table 3).

Chlamydia PAF

Among non-black women without endometriosis, the unadjusted PAF for HSG TFI was 21.8% (95% CI 10.1–33.3%). After adjustment for age, clinic location, and household income in our multivariable model, the aPAF of chlamydia in HSG TFI in these non-black women was 19.8% (95% CI 7.7–32.2%) (Figure 2).

Discussion

In these women with TFI and non-TFI infertility, we measured prior chlamydia using the enhanced Pgp3 ELISA — a chlamydia serological assay with better sensitivity and comparable

specificity to other MOMP-based serological assays — and estimated the PAF of TFI due to chlamydia. While other studies have characterized chlamydia seropositivity in women with TFI(5, 17), our study is notable because we found a significant association of Pgp3 seropositivity among some women in our stratified analysis by racial group. Among non-black women without endometriosis, Pgp3 seropositivity was independently associated with the odds of TFI and in these women, 20% of TFI was attributable to chlamydia.

In the context of the imperfect sensitivity and specificity of many chlamydia serological assays for detecting prior chlamydia(8), with a more sensitive serological assay, we found that Pgp3 seropositivity was associated with the unadjusted odds of TFI among all women combined, and also among non-black women. We also found an overall chlamydia PAF of 20% in non-black women without endometriosis which is higher than our prior study evaluating the unadjusted PAF of HSG-diagnosed TFI due to chlamydia, where 11% [95% CI -3 to 23%] of TFI was attributed to chlamydia when chlamydia seropositivity in non-black women was assessed with a reference of positivity on a combination of the commercial Medac IgG MOMP ELISA or the research-based EB ELISA assays(9), although this was not a statistically significant association. Pgp3's higher sensitivity compared to the Medac MOMP assay may have resulted in the association we observed in this study compared to the lack of association with our previous reference assay(8). Using prior serologic data and modeling to account for imperfect sensitivity and specificity of chlamydia serological assays, UK investigators estimated the PAF of TFI from chlamydia in a Dutch population from 1992–2003 to be 45% [28–62%] (6). Price et al. applied multi-parameter evidence synthesis of available non-serologically based data sources and estimated, using modelling, that the TFI chlamydia PAF in England in the early 2000s was 29%

(95% CI 9%–56%) and pooled modeled estimates from observational case-control studies found the TFI chlamydia PAF ranged from 28–60%(7). Another study using modeled distributions of women in the UK between 1985 and 1995 with differing whole-cell immunofluorescence chlamydia antibody titers estimated that the minimum PAF of TFI from chlamydia was 28% (95% CI 7% to 50%) and maximum PAF was 46.8% (95% CI 23% to 64%)(18). Our 20% estimate using Pgp3 is higher than our previous estimate using different assays but lower than the previously published modeled estimates. Taken together, the estimates suggest that a minority of TFI is caused by chlamydia, the most common reportable STI in the US and worldwide(3, 19). While gonorrhea has also been implicated as a cause of TFI, gonorrhea is generally less common than chlamydia(3). When both of these STIs are considered together, they are estimated to be responsible for less than half of all PID, and presumably also less than half of TFI cases(20).

Interestingly, we found the association of Pgp3 chlamydia serology with TFI varied by the presence or absence of endometriosis. Endometriosis has been associated with infertility and obstruction of the fallopian tubes possibly due to anatomical distortions due to adhesions and fibrosis(21, 22). Although chlamydial infection has been linked with endometriosis in one paper, the data for this association are poor and the mechanism for this association is not clear(23, 24). Because we did not see a significant association between Pgp3 serology and TFI among women with endometriosis, we might speculate that among these women, the association between chlamydia and TFI may be obscured by an association between endometriosis and TFI. As with our previous study(9), there was no observed association between chlamydia seropositivity and HSG-diagnosed TFI among black women. Among these women, we did

interestingly observe a negative, although non-significant, association of Pgp3 seropositivity and TFI, noting that women with TFI had lower absolute numeric Pgp3 seropositivity than controls. However, for black women in whom we might most expect to see chlamydia seropositivity (women with a history of chlamydia, women with PID history, and women with both), we did see positive, although non-significant, associations between chlamydia serology and TFI. We may not have seen a positive association between seropositivity and TFI outcome among black women because of possible higher rates of prior chlamydia in black women in the control group through mechanisms including increased frequency of uncomplicated chlamydial infections and repeated undetected chlamydial infections or undetected PID infections. It is possible that subsetting our analysis to women whom we presumed to be at higher risk for chlamydia infection than the general study population, such as those with a prior STI or PID history, may have excluded women with positive serology because of uncomplicated STIs. This exclusion may have resulted in a non-statistically significantly positive association in black women where it had previously been non-significantly negative.

An additional perspective on the seropositivity of these black women and possible limitation of our study is that black control women may have been misclassified and may have actually had TFI that was not evident on HSG, a less sensitive TFI test compared to laparoscopy(25). One study that found differing sensitivities of HSG for tubal disease comparing women without risk factors for tubal disease to women with risk factors suggested that an HSG reading could be affected by a woman's medical history and may not be simply based on blinded test results(26). In addition, there is evidence that chlamydia may also impair fertility through mechanisms other than overt tubal damage. Steiner et al. observed that infertile women with patent tubes

were less likely to conceive if chlamydia seropositive(4). Thus, our study may have been improved by the inclusion of fertile controls to address the potential misclassification bias of subclinical TFI or non-TFI chlamydia-related infertility in women considered to be controls. However, the higher proportion of chronic pelvic pain and suggestion of higher PID among black case women compared to black control women in our study argues against misclassification. Higher background chlamydia seropositivity in black controls, which is in accordance with higher chlamydia prevalence in black women(3), may be the underlying reason for a lack of association of chlamydia seropositivity with TFI in black women.

Our findings suggest that the Pgp3 assay might be a more reliable approach to differentiate prior chlamydia from a lack of prior chlamydia in non-black women with TFI compared to the commercial and research assays used in our previous analysis. It is important to note that while the enhanced Pgp3 ELISA has better or equivalent sensitivity than existing serologic assays(11, 12), any serologic assay for chlamydia has inherent challenges. Chlamydial antibodies can wane over time. Although chlamydial Pgp3 antibodies detected with the enhanced Pgp3 assay have been documented to remain positive in the majority of, but not all, women for 12 years independent of reported reinfection(12), other studies found that detection of chlamydial antibodies using the Medac MOMP(27) and indirect Pgp3 ELISA(8) declines within the first few years after initial infection. Additionally, not all women even mount an antibody response after an initial chlamydial infection(27). An additional limitation in our study is that there is no gold standard test for past infection that we could have assessed as a reference standard. This is particularly notable because we were unable to detect all reported chlamydial infections with Pgp3, resulting in possible misclassification.

Even in the context of the limitations of the enhanced Pgp3 assay, we estimate that 20% of TFI among non-black women without endometriosis in these clinics is attributable to chlamydia. Given the limited sensitivity of Pgp3 and chlamydia serologies in general, this figure may be an underestimate. With the differential association we observed by race, our data suggest that stratified analyses of TFI in the US by race should be done when assessing the contribution of chlamydia and other STIs to this pathology. With the lack of association of Pgp3 seropositivity with TFI among black women, additional research should be done to understand chlamydia risk in TFI for these women and whether it may cause infertility through other mechanisms. Although serologic tests for previous chlamydia can provide some estimate of previous disease and are needed to identify outcomes of chlamydial disease(28), they are imperfect and there is need for more accurate biomarkers that can predict ascension of chlamydia and other STIs into the upper genital tract predisposing to PID and TFI in all women.

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