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Chlamydia trachomatis today: treatment, detection, immunogenetics and the need for a greater global understanding of chlamydial disease pathogenesis

Deborah Dean, MD, MPH[Director][Children's Global Health Initiative][Professor of Medicine]
University of California at San Francisco School of Medicine Faculty, UCSF and UCSF/Univ. of Calif. at Berkeley Joint Graduate Group in Bioengineering Senior Scientist, Center for Immunobiology and Vaccine Development Children's Hospital Oakland Research Institute 5700 Martin Luther King Jr. Way Oakland, CA 94609

Summary

Chlamydia trachomatis is an important human pathogen causing a myriad of severe and debilitating diseases. While antibiotics have been a mainstay of treatment, there is increasing evidence for potential drug resistance, re-infection and persistent infections that require a reevaluation of treatment strategies. A critical need to address these issues will be a rapid, sensitive and cost-effect diagnostic that can be used for global screening, treatment and test-of-cure of infected individuals instead of empiric therapy that not only drives drug resistance but is not cost effective. This type of diagnostic would allow clinicians and researchers to evaluate the true incidence and prevalence of chlamydial infections in both developed and developing countries. There is extremely limited data on chlamydial sexually transmitted diseases (STD) in many developing countries including those in Central and South America. In addition, advancing our understanding of chlamydial disease pathogenesis will required an evaluation of host genetic susceptibility to infection and sequelae. We provide preliminary data on rates of chlamydial STDs and host genetic factors that predispose to infection among adolescent pregnant and non-pregnant commercial sex worker populations residing in Quito, Ecuador.

Introduction

Chlamydia trachomatis (*Ct*) is a human pathogen and member of the family Chlamydiaceae that is comprised of the genus *Chlamydia* and nine species, including *Ct* (1). *Ct* is an obligate intracellular Gram-negative bacterium and is responsible for trachoma, a prevalent blinding disease found in tropical developing countries, and sexually transmitted diseases (STD). With over 92 million cases occurring worldwide each year, *Ct* is the leading global cause of bacterial STDs (2). The spectrum of these STDs range from ophthalmia neonatorum and pneumonitis in the infant to urethritis, cervicitis, pelvic inflammatory disease (PID), proctitis, reactive arthritis and inguinal lymphadenitis among adolescents and adults. *Ct* has also been implicated in invasive squamous-cell carcinoma of the uterine cervix (3-5) and as a complicating factor in HIV-1 infection and transmission (7). Approximately 75% of women and 50% of men have asymptomatic urogenital infections (8-10), which represents a huge population of untreated individuals who can unknowingly transmit the organism. Furthermore, protective immunity appears to be short-lived. *Ct* antigens do not readily enter the class I or class II antigen presentation pathway to stimulate

Corresponding author: Deborah Dean, MD, MPH 510-450-7655 (O) 510-450-7910 (fax) ddean@chori.org.

an immune response (11), which may account for these asymptomatic *Ct* infections that are so widespread today.

The development of an effective vaccine would be invaluable for decreasing the worldwide morbidity from *Ct* infections. However, chlamydial vaccine development continues to pose a challenge due in part to an incomplete understanding of the human immune response to the bacterium (12, 13). Further, while many *Ct* ocular and urogenital infections are thought to be effectively treated by antibiotics, follow-up studies have revealed that many individuals develop re-infection, treatment failure or *Ct* persistence (14-22).

Antibiotic resistance

There is also the disturbing development of what appears to be emerging *Ct* drug resistance to azithromycin (23, 24). In trachoma endemic populations that are receiving mass treatment with azithromycin, other pathogens, such as *Streptococcus pneumoniae* and *Shigella* spp., have also developed resistance to this drug (25, 26). This is of concern because of the importance of these drugs in treating severe infections with these species especially in developing countries. In addition, it has recently been reported that *Chlamydia suis*, a species that is very closely related to *Ct* and infects the intestinal tract of pigs, has acquired a tetracycline resistance transposon probably from another gut pathogen, *Helicobacter pylori* (27). This has occurred from animal feed that has been laced with tetracycline in order to decrease infectious diseases among livestock. These are the warning signs of antimicrobial pressure on chlamydial pathogens that may result in further drug resistance and an inability to eradicate or control infection and disease in humans, especially with coinfections of the same or different *Chlamydia* spp. where transfer of antibiotic transposons may occur (57,69). Also, the overuse of drugs for empiric treatment of presumed *Ct* infections and the associated costs are issues that can be addressed if a rapid, sensitive and cost effective diagnostic is available. This type of diagnostic could also be used to develop appropriate treatment regimens. Therefore, effective screening and treatment strategies are of significant importance to increase prevention and reduce morbidity from *Ct* infections and the serious clinical sequelae.

C. trachomatis detection

While there are commercial nucleic acid amplification tests (NAATs) on the market to detect *Ct* [ProbeTec ET (BD); APTIMA Combo 2 and APTIMA assay (Gen-Probe); Amplicor PCR Assay (Roche); Hybrid Capture 2 CT-ID DNA Test (Digene)] (28-30) with a level of detection (LOD) of 1-10 copies of plasmid or *ompA* gene (31), they are expensive and require extensive investment in equipment and technical expertise. In addition, the concordance for one NAAT in confirming the sensitivity of another ranges from 71.5% to 99.4% (32, 33). In general, the sensitivity and specificity are 80 to 97% and 91 to 99%, respectively, depending on the sample source (31). In addition, NAATs only target one or two loci: the multi-copy cryptic plasmid, 16S rRNA or the single copy major outer membrane protein (MOMP) gene (*ompA*). Yet, some *Ct* strains do not contain the plasmid (34). Recently, both the Roche and BD tests were unable to detect strains in Sweden that contained a 377 base pair (bp) deletion in the exact region of the plasmid where the PCR primers had been designed (35). Finally, the NAATs cannot discriminate strain types. Strain typing capabilities are important for detecting invasive vs. non-invasive strains of *Ct*, the former of which are known as lymphogranuloma venereum (LGV) strains and require a much longer treatment interval for infection eradication (36). The combination of a rapid diagnostic with strain typing capabilities would be invaluable for treatment decisions, especially given the increasing worldwide rates of *Ct* infections and LGV strains in particular (37-39), and for tracking drug efficacy. While the new Abbott m2000 has an LOD

of 20 plasmids (75 min hands-on time with results in 4.5 hours) (40), the required equipment, cost, and inability to discriminate strain types are major limitations.

C. trachomatis in Latin America

Introduction

The lack of cost effective diagnostics for *Ct* infections has curtailed our ability to screen populations throughout the world to obtain a more realistic picture of incident and prevalent *Ct* rates, especially in developing countries. For example, there are few studies among countries in Latin America on *Ct* STDs, and these have primarily been conducted in Ecuador, Honduras, Mexico, Nicaragua, Panama, and Peru (41-55). The majority of these investigations were conducted before the availability of NAATs and relied on culture, direct fluorescent antibody (DFA) or serology, which are much less sensitive. However, some recent studies using NAATs have documented, in general, high rates of *Ct* infection of up to 25%, especially among female sex workers (FSW). These findings point to the need for rapid point-of-care diagnostics, educational control programs, and appropriate treatment with follow up guidelines. Nicaragua has been one country in which a few surveillance studies have been performed. In a study by Gorter *et al*. (45), the authors not only tested for *Ct* and other STDs among sex workers and their contacts but provided a voucher system to promoted decreasing risky behavior.

C. trachomatis in Ecuador

Another country with limited surveillance is Ecuador. The first study was published in 1955 and documented rates as high as 55% among FSWs. In 1986, using DFA on samples from over 300 individuals, researchers found that the prevalence of *Ct* infection was 53.4% among FSW and 34.5% among partners but only 1.6% among pregnant women (53). Thus, more current information about the prevalence of *Ct* infection in Ecuador and other Latin American countries would provide a better understanding of the existing burden of chlamydial disease in the region. We have conducted two studies in the capital, Quito, to evaluate prevalence rates among pregnant adolescents and non-pregnant FSWs. The purpose of these studies was to raise regional awareness about *Ct* STDs and pave the way for the implementation of appropriate screening and treatment interventions in Ecuador. In the first study, we found that only 10 (6.6%) of 150 pregnant adolescents were positive for *Ct* by the commercial Amplicor PCR test (Roche) and in-house PCR that was used to resolve samples that were either indeterminant or were shown to be inhibited using the Roche Internal Control plate as we have previously described (56, 57). Our rate was higher than in the 1989 study, which is consistent with the higher sensitivity of NAATs compared to DFA.

In the second study, which is underway, we have obtained over 1000 cervical samples from adolescent and adult women (ages 13 to 26 years) from clinics serving FSWs in Quito, Ecuador, a country in which prostitution is legalized. These women are being followed every three months to determine the incidence and prevalence of *Ct* and other STDs. Females who test positive for *Ct* are treated as per standard care. In addition, each woman is tested for HIV-1. So far, we have found a prevalence rate of 24% for *Ct* and 0.1% for HIV.

Immunogenetics

Our group, in addition to screening for *Ct*, has been interested in understanding the host-pathogen interaction in relation to disease and the importance of inflammation in *Ct* STDs. We have previously used human genetic typing for 51 single nucleotide polymorphisms (SNP) in 36 genes associated with inflammation to identify those SNPs that may predispose to the severe sequelae of trachoma, referred to as trachomatous trichiasis (TT) among our trachoma populations (58). Using Logic Regression, we found individual SNPs for tumor

necrosis factor apha (TNFA), lymphotoxin alpha (LTA) and vascular adhesion molecule-1 (VCAM1) that were significantly associated with TT compared to individuals without any evidence for trachoma. More importantly, the synergistic SNPs of TNFA (-308G), vitamin D receptor (VDR; intron G), Interleukin (IL)4 receptor (R; 50V), and Intracellular adhesion molecule-1 (ICAM1; 56M) minor allele increased the odds of TT by 13.5. Other studies in trachoma populations have shown an association of TNFA (308 GA), LTA (+252 GG), and inhibitor of kappa light chain gene enhancer in B-cells-like (IKBL, 263 TT) SNPs with trachomatous scarring (TS) or TT (59-62). Matrix metalloproteinase 9 (MMP-9) was found to be associated with a lower risk of TT (63). These studies suggest a role for SNPs in the disease pathogenesis of trachoma and possibly the pathogenesis of *Ct* STDs given the pathologic similarities between conjunctival and fallopian tube scarring.

We applied the same approach as above, and, in preliminary studies, have found a significant association between *Ct* infection and SNPs in TNFA and ICAM1 compared with uninfected FSWs in Quito who represent the indigenous population. Until recently, SNP data only existed for human leukocyte antigen (HLA) types among STD populations infected with *Ct*. DQA*0101 and DQB*0501 were found to be associated with tubal infertility (TFI) among Nairobi FSW while DQA*0102 was negatively associated (64). This finding contradicts a Finish study where QA*0102 and DQB*0602 were associated with TFI (65). But, both studies used serology to diagnose Ct, which is imprecise. Also in Nairobi, DQA1*0401 and DQB1*0402 were associated with high antibody titers to CHsp60, but not with PID (66). These results represent two populations and limited typing (no DR loci and limited alleles or SNPs in inflammatory genes) that are not predictive. In a recent study by Morré *et al.* (67), the authors investigated the role of the Asp299Gly polymorphism in the Toll Like Receptor (*TLR) 4 g*ene and association with tubal pathology and found no significant association. However, in another study by Morré *et al*. (68), women with two or more SNPs among the pattern recognition receptor genes (TLR9, TLR4, CD14, and CARD15/NOD2) had an associated increased trend in the data for tubal pathology compared to women with less than two SNPs.

Conclusions

While candidate and genome wide association studies are still in their infancy for *Ct* ocular and sexually transmitted diseases, these approaches will provide a more comprehensive understanding of the role of host single nucleotide polymorphisms (SNP) in disease susceptibility, reinfection and persistence. In addition, pairing theses studies with improved, rapid diagnostics and strain typing will accelerate what we can learn globally about the pathogenesis of *Ct* diseases.

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