Absence of chlamydial deoxyribonucleic acid from testicular and epididymal samples from men with obstructive azoospermia

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Objective: To identify *Chlamydia trachomatis* DNA by polymerase chain reaction in the upper genital tract of men with obstructive azoospermia compared with men seeking vasectomy reversal.
Design: Case–control study.
Setting: Tertiary referral center, Aberdeen Royal Infirmary, Aberdeen, United Kingdom.
Patient(s): Cases were men with idiopathic obstructive azoospermia, and controls were men with azoospermia secondary to vasectomy.
Intervention(s): *Chlamydia trachomatis*–specific DNA test by polymerase chain reaction on testicular and epididymal biopsy samples, as well as epididymal aspirate.
Main Outcome Measure(s): Presence of *Chlamydia trachomatis* DNA.
Result(s): We did not detect the presence of *Chlamydia trachomatis*–specific DNA by polymerase chain reaction in the epididymis or testis of 36 asymptomatic men with obstructive azoospermia (14 cases, 22 controls).
Conclusion(s): Our hypothesis that unrecognized, asymptomatic chlamydial infection will lead to complete bilateral obstruction of the male genital tract remains unproven. (Fertil Steril® 2010;93:833–6. ©2010 by American Society for Reproductive Medicine.)

Key Words: Chlamydia, male infertility, azoospermia

Subfertility affects one in seven couples. In half of them a male factor acts as a sole or contributing cause (1). In the majority of these cases the etiology remains unexplained (2, 3). *Chlamydia trachomatis* (CT) is the commonest bacterial sexually transmitted infection in the United Kingdom (UK) (4) and can result in infections in the urethra, epididymis, and prostate (5, 6). *Chlamydia trachomatis* infection (including chronic infection) is frequently asymptomatic, and standard microbiologic tests may fail to reveal the pathogen owing to false-negative results (5, 7–9). Some studies have demonstrated the presence of CT DNA in the semen (10) or prostate (11), when first-void urine samples were negative.

It has been suggested that the immune response to an infecting CT strain is influenced by host genetic determinants, the route of infection, the host endocrinologic state, the infection load, and antibiotic therapy. The combination of all of these factors can result in several clinical outcomes, one of which is a persistent chronic state of infection (12). Chlamydial antigens have been detected in specimens that could represent the long-term consequences of female genital tract infection (e.g., fallopian tube specimens removed for ectopic pregnancies and endometrial and ovarian biopsies) (13).

Wide variation exists in the literature on the prevalence of chlamydial antigen (1%–40%) among asymptomatic men with a longstanding history of couple infertility. In a recent study on the prognostic value of basic semen analysis, microbiologic tests revealed a high proportion (43%, 95% confidence interval 3%–76%) of CT-specific DNA in semen from men with azoospermia (14). This suggests a possibility that chronic progression of CT in the male genital tract could lead to azoospermia, but the precision of the results is questionable owing to the small sample size ($n = 7$). In animals, CT epididymo-orchitis has been produced by urethral inoculation and intraluminal spread (15). An excisional testicular biopsy from men with less than 1×10^6 sperm revealed that 50% had normal spermatogenesis, and 12% of these men had a history of male accessory gland infection (16).

We hypothesized that chronic unrecognized chlamydial infection in the male genital tract could lead to obstruction in the male genital tract, and we aimed to identify CT DNA by polymerase chain reaction (PCR) in the upper genital tract of 36 azoospermic men. To detect epididymal presence of CT DNA epididymal aspirates were obtained in addition to testicular biopsy.

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MATERIALS AND METHODS

Cases involved 14 azoospermic men with normal karyotype, FSH level less than 12 IU/mL, with no history of ejaculatory disorders, normal results on scrotal examination, and testicular biopsy revealing evidence of sperm suitable for intracytoplasmic sperm injection, whereas 22 men with postvasectomy azoospermia served as controls. All men were scheduled to undergo a surgical sperm retrieval procedure. We did not limit our study to cases with previous sexually transmitted infections (STI) because the majority of cases of CT infection remain asymptomatic. All patients had a detailed history and examination as per our clinic protocol. Ultrasound scan was only carried out if there was a clinical abnormality. Cystic fibrosis (CF) mutation screening was offered to all cases. Controls were only offered CF screening if the vasectomized men had not fathered any children before vasectomy. Couples in which both partners had a family history or were carriers of the mutation were also offered CF screening by protocol. Results from microbiologic analysis of semen as well as serologic tests for HIV and hepatitis B and C were negative among study subjects. All study subjects had a mid-stream specimen of urine for culture and *Chlamydia* examination. *Chlamydia trachomatis* DNA testing was carried out on testicular biopsy and epididymal aspirate samples obtained from the surgical sperm retrieval procedure. Where sperm was not retrieved from the epididymal aspirate an epididymal biopsy was carried out. Table 1 shows the number and site of samples tested among cases and controls. Epididymal fluid samples were aspirated with culture media used in assisted reproduction (Medicult, Jyllinge, Denmark) at a dilution of 1:7, whereas biopsy samples were collected dry in sterile containers. All samples were stored at −80°C. The study was approved by the Grampian Research Ethics Committee. We did not perform a formal power calculation because we were unable to ascertain the prevalence of chlamydia among azoospermic men from the literature. Existing reports included prevalence data on either the general population or specific subgroups (e.g., infertile men, army recruits) (17). In a subgroup analysis among seven men with azoospermia the prevalence was found to be high (42.9%) (14). Although this suggests a high prevalence and is in support of our hypothesis, the precision of these data is questionable.

TABLE 1

Baseline characteristics of participants.

Characteristic	Cases (n = 14)	Controls (n = 22)
Age, median (range) (y)	36 (34–41)	42 (40–47)
History of previous STD	0	1
Genitourinary symptoms	1	0
Karyotype 46,XY	14	22
FSH level (<12 IU/L)	14	22
Cystic fibrosis mutation	0	1

Note: STD = sexually transmitted disease.

Sripada. Chlamydia and obstructive azoospermia. Fertil Steril 2010.

Deoxyribonucleic acid was extracted from all testicular and epididymal specimens with the QIAamp DNA microkit (Qiagen, Crawley, UK) as per the manufacturer's instructions. Polymerase chain reaction amplification of human 18S ribosomal RNA (rRNA) gene was carried out for all samples to ensure efficient DNA extraction. Primers 5′-CGT CTG CCC TAT CAA CTT TC (forward) and 5′-GGT GCC CTT CCG TCA AT (reverse) amplified a 867-bp fragment and were based on the human 18S rRNA gene sequence (GenBank accession number X03205). Polymerase chain reaction conditions for 18S amplification comprised an initial denaturation step at 94°C for 4 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and elongation at 72°C for 1 minute. Amplification cycles were followed by a final elongation step at 72°C for 7 minutes. Human DNA previously extracted from a fetal testicular sample was used as template in positive control reactions and distilled water in negative controls. Amplification of CT ompA gene was carried out as described by Jurstrand et al. with primers P1 5′-ATG AAA AAA CTC TTG AAA TCG G (forward) and OMP2 5′-ACT GTA ACT GCG TAT TTG TCT G (reverse), which amplify approximately a 1.1-kb fragment. Polymerase chain reaction conditions for ompA amplification comprised an initial denaturation step at 94°C for 10 minutes and 40 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 1 minute, and elongation at 72°C for 1 minute and 30 seconds, followed by a final elongation step at 72°C for 10 minutes (18). *Chlamydia trachomatis* DNA, previously extracted from cultured CT (serovar K) or from a first-void urine sample that was positive in a routine diagnostic BD ProbeTec assay (Becton Dickinson, Cowley, Oxford, UK), was used as template in positive controls and distilled water in negative controls. Extractions of DNA were carried out in a class I safety cabinet, and pre- and post-PCR manipulations were performed in separate rooms to avoid contamination. Polymerase chain reaction reactions contained forward and reverse primers (250 nM), MgCl2 (1.5 mM), 2′-deoxynucleoside 5′-triphosphates (200 μM, Amersham Pharmacia Biotech UK, Little Chalfont, UK), and Taq polymerase (5 U) plus buffer (Bioline, London, UK). Template DNA was one tenth of the reaction volume in 18S amplification and one fifth in ompA amplification. Polymerase chain reaction was performed on a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems, Warrington, UK), and PCR products were detected by electrophoresis on agarose gels followed by staining with ethidium bromide and ultraviolet transillumination.

RESULTS

The median (interquartile range) age of cases and controls was 36 (34–41) years and 42 (40–47) years, respectively. The clinical history and findings among cases and controls are summarized in Table 1. All study subjects except one man in the control group denied previous STI. All extracts