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Diagnosing *Chlamydia Trachomatis* Urinary Tract Infections – Preliminary Report

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A – research concept and design; **B** – collection and/or assembly of data; **C** – data analysis and interpretation; **D** – writing the article; **E** – critical revision of the article; **F** – final approval of article; **G** – other

Abstract

Background. *Chlamydia trachomatis* is mentioned among the etiologic factors for urinary tract infections. Chlamydias are parasites inside a cell. A very significant problem of *C. trachomatis* infections is their asymptomatic character. The most frequent infections caused by these bacteria are inflammations of the urethra and bladder; of the vagina, cervix, vaginal cavity and adnexa in women; and of the epididymis, testicles and prostate in men. In the diagnosis of *C. trachomatis* infections, the following methods are used: immunofluorescent techniques, immunoenzymatic assays, serological examinations and genetic techniques (for example PCR).

Objectives. The aim of the study was to detect *C. trachomatis* among patients with symptoms of non-gonorrheal urethritis using diagnostic serologic methods and direct immunofluorescence. The purpose was to assess the connection between the incidence of urinary tract infections caused by *C. trachomatis* and symptoms that patients report as well as other data from interviews.

Material and Methods. Blood serum and urethral smears were taken from each of 57 patients. The ELISA method was used to mark specific IgG and IgGcHSP60 anti-chlamydia antibodies in the blood serum. In the urethral smear, antigens were marked using the direct immunofluorescence method.

Results. Evidence for urinary tract infection caused by *C. trachomatis* was found in 15.79% of the examined patients using the immunofluorescence method. In the blood serum, positive results for IgGcHSP60 were obtained in 17.54% of the patients and for IgG in 8.77%.

Conclusions. The studies carried out so far suggest that *C. trachomatis* has a significant role in the etiology of urethritis in adults and children. Other serological tests should be conducted in all the patients in order to study the immune responses in infected individuals and to confirm *C. trachomatis* infection using genetic methods such as PCR (**Adv Clin Exp Med 2015, 24, 3, 441–445**).

Key words: NGU, *Chlamydia trachomatis*, IgGcHSP60.

Chlamydia trachomatis belongs to a group of sexually transmitted microorganisms. According to data from the World Health Organization (WHO), more cases of sexually transmitted disease (STD) are caused by *C. trachomatis* than by any other bacterial pathogen. These infections have become a major public health issue all over the world. This pathogen shows viral features, i.e. a small size and an intracellular life cycle. What is more, it has the ability to produce its own adenosine triphosphate (ATP) and displays bacterial features as well, for example: the cell wall lacks muramic acid but contains lipopolysaccharide (LPS); the cells have 2 nucleic acids (DNA and RNA), organelles and antibiotic resistance [1].

One of many infections caused by *C. trachomatis* serotypes D to K is non-gonorrheal urethritis (or nongonococcal urethritis, NGU). Among the symptoms described by patients suffering from NGU are pain during urination and a burning sensation of the urethra [1, 2]. Infection of the urethra often shows the features of an inflammatory condition, which can be a result of contagious and non-contagious factors. Clinical symptoms, when they occur, are pus/mucus or pus discharge, discomfort, problems with urination and/or an

itching sensation in the urethra. Asymptomatic infections are also very common among both men and women.

The incidence of chlamydia is very high among people aged 25 and under [3]. Non-gonorrheal urethritis is caused by *C. trachomatis* in 15% to 40% of the cases; however, the frequency incidence depends on the age group, and infections rarely occur among older men. Complications of NGU among men infected by *C. trachomatis* include inflammation of the epididymis and Reiter's syndrome. The treatment of urethritis should be initiated immediately after the pathogen is established. Azithromycin and doxycycline are very efficient in the treatment of chlamydia [3, 4]. Among women, *C. trachomatis* infections may cause severe after-effects, the most serious of which are pelvic inflammatory disease (PID), ectopic pregnancy and infertility. The US Centers for Disease Control and Prevention (CDC) recommend annual screening to identify chlamydia among sexually active women from the age of 25 onward. *C. trachomatis* infections of women's urogenital systems can be diagnosed by urinalysis or cytopathology of the urethra, cervix uteri or vagina. *C. trachomatis* in men's urethras can be detected by examination of the urethra or by urinalysis. A nucleic acid amplification test (NAAT), cell culture, direct immunofluorescence (DIF), EIA (enzyme immunoassay) and nucleic acid thermodynamics tests are used to detect *C. trachomatis* in cytopathology of men and women [3, 4]. Since chlamydia is a sexually transmitted microorganism, the patients' sexual partners need to be informed and treated.

To minimize the transmission of the microorganism, patients treated for NGU should refrain from sexual activity for seven days after a single dose treatment or until the last day of a 7-day treatment, if the symptoms disappear. Clinical symptoms alone, without microbiological tests of the urethritis, are not enough to justify re-treatment. Infected patients may suffer from afflictions and complications that can last up to 3 months, such as chronic inflammation of the prostate, chronic pain in the perineum, phallus or pelvis, discomfort and pain during or after ejaculation. Patients infected by chlamydia are exposed to a high risk of re-infection within 6 months of the end of treatment; tests should be repeated within 3 to 6 months after treatment, regardless of patients' assertions that their sexual partners have been treated. Objective symptoms of urethritis should be visible before beginning antibiotic therapy. Symptoms of chronic or recurring urethritis most often appear among people who failed to complete the first treatment, who were inadequately treated or who were re-infected by a sexual partner who was not treated [3].

The major outer membrane protein (MOMP) of chlamydia and chlamydial heat shock proteins are among the species-specific antigens. MOMP is a protein whose molecular weight is approximately 40 kDa; it comprises 60% of the weight of the outer membrane proteins. It is built of four variable hydrophilic domains exposed outside the cell membrane and surrounded by 5 hydrophobic domains occurring on the inner surface. MOMP acts as a structural protein and is involved in the differentiation of the chlamydial developmental cycle. It is a protein with highly immunogenic properties, containing epitopes specific for the genus, species, subspecies and serotype. Chlamydial heat shock protein antigen (c-HSP60), or heat shock protein, whose molecular weight is 60 kDa, is moderately immunogenic in comparison to the poorly immunogenic smaller protein of a molecular weight of 12 kDa [1, 5, 6]. They are invisibly present on the surface of the membrane, and antibodies specific for these proteins do not bind to live chlamydial elementary bodies (EB) of *C. trachomatis*. *C. trachomatis* is a potent immunogen, stimulating the immune processes of microorganisms. In the course of *C. trachomatis* infection, non-specific, specific, humoral and cellular response mechanisms are involved. c-HSP60 causes delayed-type hypersensitivity reactions, which contributes to irreversible consequences in the course of infection with *C. trachomatis*. When a patient has positive results in a test for IgG, this is an indication of past *C. trachomatis* infections. If tests for both IgG and IgGcHSP60 are positive, there is a high probability of *C. trachomatis* induced tubal factor infertility (TFI) [5–7].

Material and Methods

The aim of the study was to detect *C. trachomatis* among patients with symptoms of non-gonorrheal urethritis using diagnostic serologic methods and direct immunofluorescence. The purpose was to assess, among patients of general practitioners, the connection between the incidence of urinary tract infections caused by *C. trachomatis* bacterium and symptoms that the patients report as well as other data from interviews.

The test group consisted of 57 adult patients (17 women and 40 men) with inflammation of the urethra, referred by family physicians. The mean age of the population studied was 40.0 ± 14.7 years. Clinical symptoms occurring in the patients were burning, itching, inflammation and leucocyturia in the urine.

Blood serum and cytopathology swabs of the urethra were taken from each patient. ELISA kits

(Medac GmbH, Wedel, Germany) were used to mark specific IgG and IgGcHSP60 anti-chlamydia antibodies in the blood serum. In the urethra smears antigens were marked by the direct immunofluorescence method, using a kit that directly detects *C. trachomatis* antigens in clinical specimens (the Pathfinder® *C. trachomatis* Direct Specimen test, Bio-Rad Laboratories, Inc., Life Science Research Group, Hercules, CA, USA).

Results

The *C. trachomatis* antigen was detected in 9 (15.79%) out of the 57 patients. The percentage of positive results for IgG and IgGcHSP60 amounted to 8.77% (5/57) and 17.54% (10/57), respectively. The results of the tests by immunofluorescence and blood immunoenzymatic methods are presented in Table 1. Serological evidence of chronic infection (IgG + and IgGcHSP60 +) was found in 5 patients; only 1 patient had positive results in all the tests.

Table 1. The frequency of *C. trachomatis* infection according to the diagnostic tests used

Results of research	IFt + (%)	IgG + (%)	IgGcHSP60 + (%)
The study group n = 57	9 (15.79 %)	5 (8.77%)	10 (17.54%)
Women n = 17	1 (5.88%)	2 (11.76%)	2 (11.76)
Men n = 40	8 (20.00%)	3 (7.50%)	8 (20.00%)

Discussion

The results of the present study were compared with the results of other authors' research results relating to the frequency of infections caused by *C. trachomatis*. Due to the low number of studies on the presence of specific anti-cHSP60 antibodies in the serum and the C-reactive protein (CRP) gene in the urine of patients with NGU, and due to a lack of research studies involving women suffering from NGU, the research discussed here refers to other *C. trachomatis* infections.

Horner et al. [6] examined 113 men (aged from 19 to 53 years) for *C. trachomatis* infection. Smears from the urethra, samples from the first urine stream and serum were taken 4 times (week 0, week 2 and week 6 and week 12), and were used as the basic materials for the research. A DIF test was used to detect the EB in the smear from the urethra. Patients with positive DIF results underwent PCR tests of their urine samples and the concentration of significant anti-cHSP60 antibodies in their serum was marked by ELISA tests. At the first visit anti-cHSP60 antibodies were detected in the serum of 32/113 patients (28.3%), and 27/113 patients (23.8%) developed chronic infection of the urethra despite the treatment.

Frej-Mądrzak et al. [8] analyzed the frequency of *C. trachomatis* antigens, the CRP gene and anti-cHSP60 antibodies among infertile women. In that study 108 patients were examined at the First and Second Obstetrics and Gynecology Clinics at Wroclaw Medical University. Urethral smears, urine samples and serum samples were taken from the patients. The presence of the *C. trachomatis* antigen in the urethral smears was tested by the direct immunofluorescence method and was found in 32/108 women (29.6%). Amongst 29/108 women (26.8%) the presence of the CRP gene was found in the urine by the nested-PCR technique. Amongst 17/108 patients (15.7%) the presence of anti-cHSP60 antibodies was found in the serum.

Hejlholti et al. [9] studied the presence of anti-cHSP60 antibodies in the serum in relation to infertility resulting from stenotic fallopian tubes (TFI) among 162 Danish women. The study group consisted of 70 women; the first control group contained women with healthy fallopian tubes (n = 92) amongst whom a subgroup was selected: the second control group containing women with healthy fallopian tubes but with anti-MOMP or anti-cHSP60 *C. trachomatis* antibodies (n = 28). A serum ELISA assay was conducted among all the women examined. The researchers noted the concentration of IgG1 and IgG3 anti-CHSP60 antibodies. In 54/70 women in the study group (77.1%) IgG1 anti-cHSP60 antibodies were found the serum, and in 43/70 (61.4%) IgG3 anti-cHSP60 antibodies were found. In the first control group the presence of IgG1 and IgG3 anti-cHSP60 antibodies were found in the serum of 16/92 women (17.4%). In the second control group IgG1anti-cHSP60 antibodies were found among 16/28 women (57.1%) and IgG3 IgG1anti-cHSP60 antibodies were found in 11/28 women (39.3%). Higher concentrations of IgG1 and IgG3 anti-cHSP60 antibodies were found in the study group compared to the first control group; only IgG1 anti-cHSP60 antibodies were present in higher concentrations among the women with TFI than in the second control group.

Karinen et al. [10] examined the relationship between infertility and the presence of IgG anti-HSP60 antibodies in the serum in the Finnish

population. The study group consisted of 146 people (94 women and 52 men) and the control group included 278 people (188 women and 90 men) aged 31 years. The antibodies in the serum were detected by ELISA assay test. The researchers found IgG anti-HSP60 antibodies in 49.6% of the women in the study group and in 41.9% of the women in the control group, and in 41.8% of the men in the study group and 44.1% of the men in the control group. This research is not enough to prove a relation between the presence of IgG anti-HSP60 antibodies category in the serum and the infertility of the population.

Choroszy-Król et al. [11] examined the prevalence of *C. trachomatis* infections among children suffering from various urinary system illnesses. The study included 195 patients (116 girls and 79 boys, aged from 4 weeks to 18 years) underwent examinations at the Department of Pediatric Nephrology at Wroclaw Medical University; 195 urethra smears and 23 urine samples were taken from the children. Direct immunofluorescence and nested-PCR tests were conducted. The presence of the *C. trachomatis* antigen was found in the urethra smears of 99/195 children (50.8%), including 64/116 girls (55.2%) and 35/79 boys (44.3%). The presence of the CRP gene was found in the urine tested by the nested-PCR method in 15/23 children (65.2%), including 8/14 girls (57.1%) and 7/9 boys (77.8%).

The prevalence of *C. trachomatis*, the CRP gene and the anti-cHSP60 antibodies among the patients with non-gonorrheal infection of the urethra was analyzed in a 2014 master's thesis by Trojgo [12]. Urethra smears, serum samples and samples of urine were taken from 61 patients, including 36 men, 19 women and 6 children. Using the direct immunofluorescence method, *C. trachomatis* antigens were detected the urethral smears in 25.0% of the men and 16.7% of the children; it was not detected in the urethra smears from the women. Anti-cHSP60 antibodies in the serum were detected by the ELISA method; significant anti-cHSP60 antibodies were found in the serum of 22.2% of the men and 10.5% of the women; anti-cHSP60 antibodies were not found in the serum from the children. By the nested-PCR method, the CRP gene was found in the urine samples of 13.9% of the men, 15.8% of the women and 16.7% of the children. In the author's own research no correlation was found between the presence of anti-cHSP60 antibodies in the serum and the presence of the CRP gene in the urine samples of patients with non-gonorrheal infection of the urethra.

In the diagnostics of non-gonorrheal urethra infections, urethral smears are routinely taken to check for the presence of the *C. trachomatis* antigen. The examination is conducted by the direct immunofluoresence test. The CDC's 2014 recommendations for Laboratory-Based Detection of chlamydia concerning the diagnosis and treatment of *C. trachomatis* infections recommend genetic tests (the nucleic acid amplification test – NAAT) of the first urine stream for men and vaginal smears for women [13].

The studies carried out so far suggest that *C. trachomatis* plays a significant role in the etiology of urethritis in adults. However, further studies are required, using genetic methods in a larger group of patients, to verify research results obtained by serological or molecular tests, to study the immune responses in infected individuals and to confirm *C. trachomatis* infection using genetic methods such as PCR.

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