Clinical and laboratory study

CHLAMYDIA TRACHOMATIS IN MEN WITH NONSPECIFIC URETHRITIS AND EVALUATION OF FOUR TESTS FOR LABORATORY DIAGNOSIS

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ABSTRACT

In a study 162 men with nongonococcal urethritis (NGU) Chlamydia trachomatis (CT) infections were investigated. CT inclusions were assayed in cycloheximide-treated McCoy cells, chlamydial elementary bodies (EB) by direct immunofluorescent antibody method (DIF), by DNA hybridization assay and by indirect microimmunofluorescent detection (IIF) of IgA and IgG antibodies in serum.

One hundred and eleven asymptomatic men were also tested and were considered as controls.

CT was isolated from 43 patients with NGU (26,5 %) and from 6 men (5,4 %) of the control group ($p<0,05$). The results obtained by DIF and DNA hybridization assay were compared with the isolations on McCoy cells, which was considered as the reference method. Sensitivity of the DIF turned out to be 87,5 % and it–s specificity 97,5 %, while the sensitivity of DNA hybridization assay was 26,5 % and it–s specificity 100 %. The positive predictive value (PPV) and negative predictive value for DIF were 92,1 and 96 %, the same values for DNA hybridization assay were 100 % and 77 %. The sensitivity of the serological tests for IgA and IgG antibodies were low (40 % and 44 %), the specificity was adequate (87,3 % and 95,8 %). PPV was 52,6 % and 78,5 % and NPV 80,7 % and 95,8 % respectively.

All patients were treated with tetracyclines or with macrolides, after treatment control tests were carried out.

KEY WORDS

Chlamydia trachomatis, NGU, laboratory tests: culture on McCoy cells, direct immunofluorescence test, DNA hybridization assay, antibody detection.

INTRODUCTION

Chlamydia trachomatis (CT) is one of the most common sexually transmitted genital pathogens (1,2,3). In men nongonococcal (NGU) and postgonococcal (PGU) urethritis are, beside epididymitis, proctitis and probably prostatitis, the most frequent diseases (4). CT is the etiological agent of 35 % to 60 % of NGU depending on the population tested and laboratory tests (1,2,5). Approximately 20 % of infections were asymptomatic.

Chlamydia are obligate intracellular parasites which infect

columnar epithelial cells. Good clinical specimen for the laboratory diagnosis of infection contains scraped epithelial cells. CT can be isolated in cell culture, which is standard method but complicated, expensive and accessible only in specialized laboratories (6) Development of a rapid, sensitive and specific test which use fluorescein-conjugated monoclonal antibodies (DIF,7) or enzyme-immunoassay (EIA, 8) made the diagnosis much simpler. Recently, DNA probes have been introduced for the detection of CT (9). Serological test (ELISA, DIF) can detect immune response to chlamydial infections (2). The aim of this study was to determine the prevalence of CT infections in men and to evaluate the diagnostic value of tests available in laboratory of the Public Health Institute Pula.

MATERIAL AND METHODS

Study population

One hundred and sixty two men with clinical evidence of NGU were studied over the period from May 1991 until April 1992. Patients were from 19 to 61 years old (mean age was 33,5 years) and were treated as outpatients in the City Hospital of Pula, Croatia. Laboratory diagnosis of chlamydial infection was done in the Public Health Institute Pula and in the Institute of Microbiology Medical Faculty in Ljubljana.

All patients suffered from dysuria, with or without discharge. In all cases urethral culture for Neisseria gonorrhoeae was negative. They were not treated with antimicrobial substances.

The control group comprised 111 men without signs of NGU. Their mean age was 28,3 years.

Specimen collection

Urethral specimens were obtained by inserting Dacron tipped swabs 2-4 cm into the urethra and withdrawing it after rotation (10). We took two urethral specimens from each patient with NGU. One specimen was placed in 2SP and preserved in liquid nitrogen for preservation until inoculation on tissue culture, the second one was used in DIF. Cells scraped from urethra were displaced directly on the microscopic slide. Smears were air dried and fixed in aceton. A third specimen was taken from only 52 patients. Swabs were placed in a buffer reagent for GEN-PROBE (GP) and stored until tested.

As an informed consent for using swabs was not obtained in the controls, we looked for a chlamydial infection in the sediment of 20 ml first-void urine (11).

Cell culture method

One day old McCoy cell monolayers were inoculated with 0,5 ml of specimen in 2SP, centrifugated at 3000 g for 1 hour and incubated for 72 hours in MEM supplemented with 10 % of fetal calf serum and 1 µg/ml cycloheximide. Typical chlamydial inclusions were observed after staining with Lugol's iodine. Specimens were considered as positive when 1 or more tipical brown stained inclusions were identified.

Direct immunofluorescence tests (DIF)

Aceton fixed smears were stained with fluorescein-conjugated monoclonal antibodies (CHLAMYSET ANTIGEN FA ORION DIAGNOSTIK) according to the manufacturer's instructions. Slides were examined with an epifluorescence microscope at a magnification of 1000x under oil immersion. Smears were taken as positive if 5 or more apple-green chlamydial particles were observed.

DNA hybridization assays (GP)

Specimens were thawed and processed according to the manufacturer's directions (GEN-PROBE PACE2). In the GP assay 1 positive and 3 negative controls were included. Shortly, in a 100 µl of each specimen, chlamydial DNA was separated into a single strand at higher temperature, and then hybridized with a specific sequences of acridinium ester labeled chlamydial ribosomal RNA probe at room temperature. During the hybridization step acridinium ester was hydrolised and the released light proportional to the amount of hybride measured as relative light units (RLU).

Indirect immunoflurescence tests

Slides with laboratory prepared antigens (serotype L1 and L2, multiplied in yolk sacs) were incubated with serum dilutions and detected with fluorescein-conjugated antihuman IgG and IgA. After washing slides were examined under an epifluorescence microscope. The results were interpreted according to following criteria.

IgA $\geq$ 1:16 and IgG $\geq$ 1:16, active infection
IgA neg and IgG $\geq$ 128, active infection
IgA $\geq$1:16 and IgG neg, active infection
IgA neg and IgG $\leq$1:64 passed infection

RESULTS

CT infection was proved by one or more tests in 43 (26,5 %) of 162 patients with NGU. The prevalent age range was 25 to 35 years (Figure 1).

Chlamydial asymptomatic infection was detected in 6 (5,4 %) men in the control group.

Test results

Out of 162 patients enrolled in the study 40 were positive by culture and 38 by DIF. Overall 35 patients were positive by both tests, 5 by culture, and 3 by DIF only. Four patients of 52 had positive GP test, 3 of them were positive by culture

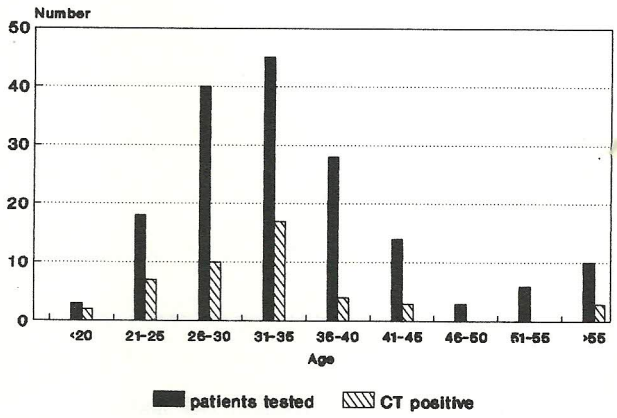

Figure 1. Age distribution of NGU patients in Pula investigated for Chlamydia trachomatis

and by DIF and 1 by culture only. Ten specimens, which were positive by culture and by DIF, were negative by GP method. Both specimens which were positive only by DIF were negative by GP test (Table1).

Table 1. Chlamydial infection detected in urethral specimens of NGU patients.

Culture	DIF	GP	No. of patients
+	+	NT	22
+	+	+	3
+	+	-	10
+	-	NT	3
+	-	+	1
-	-	-	1
-	+	NT	1
-	+	-	2
-	-	NT	84
-	-	-	35

NT = not tested

IgA antibodies were detected in 19 of 98 sera. Eleven patients had positive urethral specimens at least by one test. Eight sera were IgA positive only and repetead urethral specimens from these patients were negative again. In 17 patients with chlamydia in the urethra IgA antibodies were not found.

IgG antibodies were detected in 42 serum samples. Four of them had titres > 1:128 (3 were positive by other tests). Lower titres were found in 38 patients, 16 of them were positive by culture method, but in 10 patients with proven chlamydial infection of urethra IgG antibodies were not detectable (Table 2).

Table 2. Immune response in CT positive and CT negative patients with NGU

Titer Ig	Positive tests on CT in urethra	Negative tests on CT in urethra	Total
IgA neg	17	61	78
IgA ≥1:16	11	8	19
IgG neg	10	45	55
IgG ≤1:64	16	22	38
IgG ≥1:128	2	2	4

The results of each test were compared with results of cell culture technique. Sensitivity, specificity, and predictive values for positive and negative tests were calculated according to standard procedure (Table 3).

Posttherapy control

Patients who had proven viable chlamydia, chlamydial antigen or chlamydial nucleic acid in urethral specimen were treated with tetracycline or with macrolides. Of the 42 positive, 35 were negative at the first control after therapy. Four patients didn't come back after first visit and we assume that the therapy was successful. Another 4 patients were positive when the control specimens were investigated, and repeated therapy was effective.

DISCUSSION

Many authors reported on nongonococcal urethritis in men caused by CT (1,2,5,10). Their results differ from 30 % to 60 % which depend on the population studied and tests used for diagnosis of chlamydiosis. In the study presented we tested 162 patients with NGU and 111 healthy men. CT was

Table 3. Cumulative values of laboratory tests for CT in the investigated 162 NGU patients.

Test	Culture +	Culture -	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
DIF +	35	3	87.5	97.5	92.1	96
DIF -	5	119				
GP +	4	0	26.6	100	100	77
GP -	11	37				
IgA +	10	9	40	87.3	52.6	80.7
IgA -	15	63				
IgG +	11	3	44	95.8	78.5	83.1
IgG -	14	69				

detected in urethral specimens of 43 (26,5 %) NGU patients, and in 6 (5,4%) men without symptoms of urethritis. These findings support the etiological role of CT in NGU patients of our population ($p<0.05$). Men included in the control group without signs of urethritis and a positive test were assumed to have asymptomatic NGU.

All chlamydia positive patients and their sexual partners were treated by tetracyclines or macrolides. Four of them were positive on control visit. Therapy had been repeated and CT was eradicated.

Eighteen out of 43 chlamydia positive NGU cases had double urethral infection. In the sediment of first-void urine Trichomonas vaginalis had been found in 15 cases. Beside CT Candida albicans had been isolated in 1 case and in 2 other cases Staphylococcus aureus and Streptococcus alfa-haemolyticus.

Comparison of different methods used for detection of chlamydia in urethral specimens showed some discrepancies, which need an explanation.

Cell culture may be considered to be the reference method. However certain objections are possible: its true sensitivity and specificity are probably less than 100 %, as sampling, transportation as well as other factors are not standardized. The three urethral specimens which were negative by culture and positive by DIF may be explained in such a way.

In the literature the comparison of DIF and cell culture methods shows a wide range of agreement (60-99 %, 12,13,14). Specificity and sensitivity are better in populations where prevalence of CT infections is high. A positive result of DIF may be interpreted differently. In certain laboratories 1 EB is considered sufficient for a positive result whereas in others 10 EBs are a criterion for positivity. On the basis of our previous experiences 5 EB per smear was chosen as a criterion.

Five specimens in our population study were positive by culture and negative by DIF. Marginal quantities of viable organisms present in specimens were low (5 specimens gave only few inclusions in cell culture) which could also contribute to the discrepancies between cultivation and antigen detection. Three cases positive by DIF and negative by culture were considered as true positive because of 5 or more typical elementary bodies in smear. The results of DIF may be false positive because of cross binding of monoclonal antibodies with protein A of Staphylococcus aureus (15). In our case sensitivity and specificity of DIF were satisfying, 87,5 % and 97,5 % respectively and positive and negative predictive values also (92,1 % and 96 %).

Lees et al (16) reported a high sensitivity (86,2 %) and specificity (99,9 %) at the Gen-Probe Assay. In the study reported only 4 out of 52 investigated specimens were positive as compared to 15 positive by culture. One of the reasons for such a low number of positive GP assays was

probably a too small quantity of CT in the specimens. Lees reported that specimens with less than 10 EB had low RLU values. It has to be assumed that the eventual technical shortcommings will be eliminated soon.

We tested also 98 sera of NGU patients on specific chlamydia IgG and IgA antibodies. Taking the results of chlamydial culture as a standard the IgA and IgG determinations have sensitivity 40 % and 44 %, specificity 87,3 % and 95,8 %. Antibodies were detectable in less than a half of CT positive patients. The reason for this differences could be that sera had been taken at an early point of infection when antibody levels were still low (less than 1:16). However chlamydial urethritis is a local infection, and it is possible that antigen stimulation is not strong enough to provoke a quickly detectable immune response in serum. Among sera tested, only 8 patients had specific chlamydial antibodies (IgA > 1:16 or IgG > 1:128) and negative all other tests. Control specimens were negative again and these titres were treated as chlamydial infection nowhere else. The similar situation we observed for the IgG antibody. Our results indicate a rather low value of serology for the diagnosis of chlamydial urethritis.

The comparison of four tests, similar to finding by others, confirms that no single technique detects all chlamydial infections. When selecting a test suitable for detection CT many factors must be taken into account (cost, transportation of samples, equipment available and experience of the laboratory staff, prevalence of infection in a particular population). A false negative interpretation might be responsible for future complications, whereas a false positive reaction means unnecessary antimicrobial therapy. Combination of two or more techniques is needed for reliable laboratory diagnosis and it is necessary to test again every specimen with disagreeing result.

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