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Antichlamydial antibodies in the serum and expressed prostatic secretion in prostatitis

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Iwona Ostaszewska-Puchalska[1 ABCDEF], Bożena Zdrodowska-Stefanow[2 ADEFG], Jerzy Badyda[3 BD], Violetta Bułhak-Kozioł[1 BD], Katarzyna Puciło[1 DG] and Barbara Darewicz[4 BD]

[1] Center for Sexually Transmitted Disease Research and Diagnostics, Białystok, Poland
[2] Department of Dermatology and Venerology, Medical School, Białystok, Poland
[3] Outpatients Urology Clinic, Białystok, Poland
[4] Department of Urology, Medical School, Białystok, Poland

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Summary

Introduction: The aim of the study was to evaluate the prevalence of anti-*Chlamydia trachomatis* (*C. trachomatis*) antibodies in serum and expressed prostatic secretion (EPS) in chronic prostatitis.

Materials and Methods: Thirty-six patients with chronic prostatitis were examined. The presence of *C. trachomatis* was determined in urethral smears and EPS. Specific antibodies were determined in the serum (IgM, IgA, IgG) and in the EPS (IgA, IgG). In the direct diagnosis of chlamydial infection, the direct immunofluorescence method and the ligase chain reaction were employed, and for the serological diagnosis, the immunoenzymatic method.

Results: *C. trachomatis* infection was detected in the urethra of 3 (8.3%) patients and in the prostatic gland of 3 (8.3%) patients; only one of these patients was found to have *C. trachomatis* in both the urethra and the EPS. In the control group, *C. trachomatis* was detected in the urethra of 1/50 (2%) of the men, but the EPS of all of them was free of *C. trachomatis*. Specific IgM antibodies were found in 7 (19.4%), IgA in 9 (25%), and IgG in 18 (50%) of the patients' serum, whereas IgAs were detected in 12 (33.3%) and IgGs in 13 (36.1%) of the patients' EPS. In the control group, anti-*C. trachomatis* antibodies of the IgG were detected in the serum of 2/35 (5.7%) of the men, whereas in the EPS neither IgA nor IgG antibodies were detected in any of these patients.

Conclusions: Serological tests of the serum and EPS are useful as a complementary method in the diagnosis of chronic prostatitis.

Key words: **prostatitis • *Chlamydia trachomatis* • IgG • IgM • IgA antichlamydial antibodies • expressed prostatic secretion**

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Author's address: Iwona Ostaszewska-Puchalska, M.D. Ph.D., Center for Sexually Transmitted Disease Research and Diagnostics, Św. Rocha 3, 15-879 Białystok, Poland, tel./fax: +48 85 7423630, e-mail: centstd@poczta.onet.pl

INTRODUCTION

Chronic prostatitis (chronic pelvic pain syndrome) is a very common urological disease[4, 19]. It is estimated that one half of the male population experiences ailments connected with this syndrome in their lifetime, while its etiology is unknown in over 90% of the cases[12].

Chlamydia trachomatis (*C. trachomatis*) infection is given as one of the many causes of chronic non-inflammatory prostatitis. The issue of *Chlamydia*'s role in the etiopathogenesis of inflammatory states of the prostate gland is still the subject of controversy[3, 18, 26]. The diagnostics of chlamydial prostatitis is very difficult owing to the possibility of contamination of the expressed prostatic secretion (EPS) with material from the urethra. In recent studies attention has been drawn to the possibility of using serological tests of the EPS[11, 25].

The aim of the present study was to evaluate the prevalence of anti-*C. trachomatis* antibodies in the serum and EPS of patients with chronic prostatitis and the possible use of serological tests in the diagnosis of prostatitis.

MATERIALS AND METHODS

Thirty-six patients aged 16–53 years (mean age 38 years) with symptoms of chronic prostatitis were referred to the Center for Sexually Transmitted Disease Research and Diagnostics by urological consulting units and the Department of Urology for diagnosis of *C. trachomatis* infection. A detailed case history including the course, nature and duration of the complaints of each patient and the treatment received was first prepared, after which material was taken for direct examination and serological tests. None of the patients had been treated with antibiotics for at least 3 months before the investigations.

The presence of *C. trachomatis* was detected in the urethral smears and prostatic secretions obtained after massage of the gland. Specific anti-*C. trachomatis* antibodies were determined in both the serum (IgM, IgA and IgG antibodies) and the EPS (IgA and IgG antibodies). The urethral smears were taken with sterile dacron-tipped swabs inserted into the patient's urethra to a depth of 2–3 cm after a night's, or at least 3 hours', retention of urine. The prostatic massage was carried out on patients after micturition in whom the absence of acute urethritis and *Neisseria gonorrhoeae* infection had been established.

The control group for the *C. trachomatis* infection tests consisted of 50 men aged 22–68 years (urethral smear detection of chlamydial antigen) and 18 men aged 20–45 years (EPS detection of chlamydial antigen and antichlamydial antibodies) without subjective and objective symptoms as regards the urogenital system, whereas the control group for the serological tests of the serum comprised 35 volunteer blood donors aged 17–50 years.

Urethritis was diagnosed when 4 or more leukocytes were present in the direct preparation and 10 or more leukocytes in the gland, in the visual field of a light microscope at a magnification of ×1000.

The urethral smears and EPS were tested for chlamydia by the direct immunofluorescence method (MicroTrak, Behring) and the ligase chain reaction (LCR; LCx, Abbott, USA). For direct fluorescent antibody test, a direct smear was made on the well in a glass slide, air-dried, fixed in acetone for 5 min, kept at 4°C and stained within 5 days. The smears were stained with direct fluorescein-conjugated monoclonal antibodies and examined under the microscope for the presence of apple-green fluorescing elementary bodies. The slides were first screened at a magnification of ×400 and confirmed at ×1000. The presence of 5 or more elementary bodies was considered to indicate a positive result.

The LCR assay was performed and evaluated according to the instructions given by the manufacturers of the LCx kit. Genital specimens were collected on the spot in LCR specimen collection tubes and stored at –20°C in transport buffer for up to 2 weeks before testing. The specimens were thawed and tested in batches. For sample preparation, the specimens were placed in a heat block at 97°C for 15 min. For DNA amplification, 100 µl of each sample and each control was transferred to a microcentrifuge tube containing a predispensed LCR mix of 4 oligonucleotide probes, nucleotides, and thermostable enzymes. The tubes were inserted into a thermocycle programmed for 40 cycles. An automated microparticle enzyme immunoassay was used to detect amplicons. Clinical specimens with an assay value equal to or greater than the cut-off were considered positive.

For determination of specific antibodies the immunoenzymatic method was used. Two kinds of tests were applied: "Chlamydia IgG EIA" by Labsystem (Finland) for the determination of IgG antibodies in the serum and "Chlamydia rELISA" by Medac (Germany) for the determination of IgM and IgA antibodies in the serum and IgA and IgG in the EPS.

Synthetic peptides derived from major outer membrane protein *C. trachomatis* serotypes C, G, E, and

L_2 were used as antigen in the Labsystem kit, while in the Medac kit, fragments of lipopolysaccharide antigen specific only for the *Chlamydia* genus were used.

The serum samples were diluted to a ratio of 1:100 (IgG antibodies) or 1:50 (IgM and IgA antibodies), whereas the EPS samples were diluted to a ratio of 1:40 (IgG antibodies) or 1:20 (IgA antibodies).

Within 1 h after arresting the reaction, the measurement was made in a BIO-TEK spectrophotometer (EL 311SX) at a wavelength of 405 nm. In the tests done with the Labsystem kit, a value equal to or greater than 20 enzyme immunoassay unit (EIU) was taken to be a positive result (with < 10 EIU negativet result, 10–19 EIU doubtful, 20–59 EIU weakly positive, and >110 strongly positive), in accordance with the manufacturer's instructions. In the case of the Medac kit, a titer equal to or greater than 1:100 (IgG antibodies) and 1:50 (IgM and IgA antibodies) was assumed positive.

This study was approved by the university ethics committee.

For the statistical analysis the U-test for 2 rates of structure was applied. A difference in cases when the level of significance was equal to or greater than 0.05 was considered to be statistically significant.

RESULTS

C. trachomatis infection was detected in 3/36 (8.3%) patients in the urethra and in 3/36 (8.3%) patients in the EPS, with, however, *Chlamydia* infection being determined in both the urethra and the EPS in only 1 of the patients. In all the patients found to be infected with *C. trachomatis*, a higher number of leukocytes in the EPS was confirmed. In the control group, *C. trachomatis* were detected in one (2%) patient in the urethra ($p > 0.1742$), whereas no *C. trachomatis* was found in the EPS of any of these patients.

Specific IgM antibodies were detected in the serum of 7/36 (19.4%), IgA in 9/36 (25%), and IgG in 18/36 (50%) of the patients (Fig. 1). In the control group, anti-*C. trachomatis* IgG antibodies were noted in the serum in 2/35 (5.7%) of the men ($p < 0.0001$). In all the patients tested, IgM and IgA antibody titers in the serum were low (1:50), which was interpreted as weakly positive. The values of the IgG antibodies in some of the cases were strongly positive (Table 1).

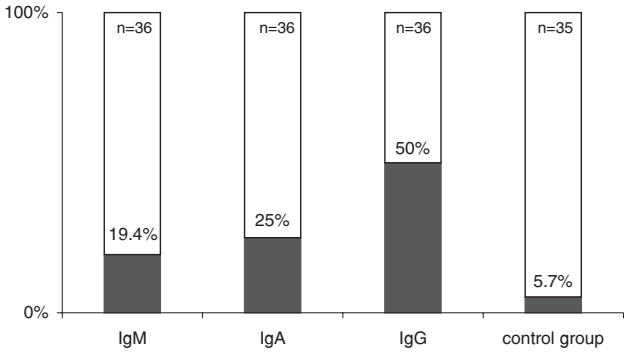

Figure 1. The prevalence of anti-*C. trachomatis* antibodies in the serum of prostatitis patients (IgM, IgG, IgA) and the control group (IgG)

The correlation between the incidence of the 3 classes of antichlamydial antibodies in the serum and the detection of the *Chlamydia* in the urethra and EPS is shown in Table 2. Of the two patients in whom *C. trachomatis* infection was detected in the urethra only, both IgG and IgA (low titer) were found in one, whereas in the other the serological tests were negative. In the patient in whom *C. trachomatis* infection of both the urethra and the prostate gland was found, 3 classes of specific antibodies were observed (low titers). Of the two patients with *C. trachomatis* infection detected in the prostate gland only, IgG (extremely high titers) and IgA (low titers) antibodies were detected in the serum of both, and in one, IgM antibodies (low titers) were also found.

In the prostate secretion, specific IgA antibodies were detected in 12/36 (33.3%) of the patients studied and IgG antibodies in 13/36 (36.1%) (Fig. 2). In the control group, neither specific IgG nor IgA antibodies were noted in any of the 18 men.

The results of the serological and direct tests for *C. trachomatis* infection in the patients whose EPS were found to contain specific IgA antibodies are presented

Table 1. Anti-*C. trachomatis* IgG antibody titers in the serum of prostatitis patients and the control group

Tested group	20–59 EIU		60–110 EIU		> 110 EIU		Total
	n	%	n	%	n	%	
Prostatitis (n = 36)	12	66.7	2	11.1	4	22.2	18
Control group (n = 50)	2	100	0	0	0	0	2

EIU – enzyme immunoassay unit

Table 2. Correlation between the prevalence of anti-*C. trachomatis* IgM, IgA, and IgG antibodies in the serum, and the positive results of direct tests in the urethra and EPS in the prostatitis group

C. trachomatis(+)	Antibodies(+)		
	IgM (n = 8)	IgA (n = 9)	IgG (n = 18)
Urethra (n = 2)	0	1	1
Urethra and EPS (n = 1)	1	1	1
EPS (n = 2)	1	2	2

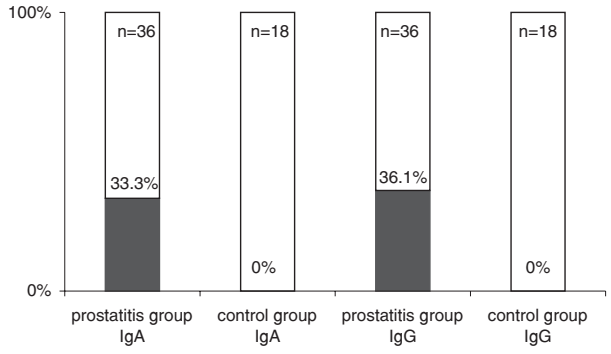

Figure 2. The prevalence of anti-*C. trachomatis* antibodies in EPS of prostatitis patients (IgA, IgG) and the control group (IgA, IgG)

in Table 3. Of the 12 patients in whom specific IgA antibodies where present in the EPS, the titers were extremely high in 6 (50%). The highest correlation was noted between the presence of IgA antibodies in the EPS and IgG antibodies in the serum (83.3%), lower correlation with IgA antibodies in the serum (58.8%), and the lowest in the case of IgM antibodies (33.3%). In two cases, *C. trachomatis* infection was detected in the prostate gland, but *C. trachomatis* infection was found in the urethra of none of the IgA cases.

Of the 13 patients in whom IgG anti-*C. trachomatis* antibodies were detected in the EPS, IgG antibodies were found in all their sera, IgA in 9 (69.2%), and IgM antibodies in 4 (30.8%). The specific IgG antibodies in the EPS of all the patients had low titers in contrast to the antibodies of this class in the serum. All three patients with detected *C. trachomatis* infection of the prostate gland also had specific IgG antibodies in the EPS (Table 4).

DISCUSSION

In the course of chlamydial urethritis in men, epididymitis and prostatitis may develop. While the role of *C. trachomatis* in the etiopathogenesis of epididymitis has been established[28], its role in prostatitis is still the subject of controversy.

The bacteriological diagnosis of chlamydial prostatitis is very difficult in view of the need to obtain suitable material for the tests (risk of contamination) and to apply the appropriate method of detecting chlamydial infection. The recent introduction of molecular methods (PCR, LCR) for the detection of *C. trachomatis* infection has considerably facilitated prostatitis diagnosis[7, 21]. The culture method most frequently used to date did not always give reliable results because of the cytotoxic properties of some of the body fluids (semen, EPS) which inhibit cell culture[13].

In our study, the LCR and direct immunofluorescence methods were employed for the diagnosis of *C. trachomatis* infection in prostate secretions in order to avoid false negative results. The problem of contamination of the EPS with urethral bacteria was solved by applying a modification of the fractional method

Table 3. Results of serological and direct tests for *C. trachomatis* in the patients with specific IgA antibodies in the EPS

Patient	Antibodies in EPS		Antibodies in serum		*C. trachomatis* antigen			Leukocytes in EPS (in the visual field)
	IgA	IgG	IgM	IgG	IgA	urethra	EPS	
1	(+++)	(+)	(–)	(++)	(+)	(–)	(–)	> 10
2	(+)	(+)	(–)	(+)	(–)	(–)	(–)	> 15
3	(+++)	(+)	(–)	(+++)	(+)	(–)	(–)	> 10
4	(+)	(–)	(+)	(–)	(–)	(–)	(–)	(–)
5	(+)	(–)	(+)	(–)	(–)	(–)	(–)	> 20
6	(+++)	(+)	(–)	(+)	(+)	(–)	(–)	> 15
7	(+)	(–)	(+)	(+)	(–)	(–)	(–)	> 15
8	(+++)	(+)	(–)	(++)	(+)	(–)	(–)	> 20
9	(+)	(+)	(–)	(+)	(+)	(–)	(–)	> 10
10	(+++)	(+)	(–)	(+++)	(+)	(–)	(+)	cover visual field
11	(+)	(–)	(–)	(+)	(–)	(–)	(–)	> 30
12	(+++)	(+)	(+)	(+++)	(+)	(–)	(+)	> 15

(+) titers 1:50–1:400, (++) titers 1:1600–1:6400, (+++) titers > 1:12800

Table 4. Results of serological and direct tests for C. trachomatis in the patients with specific IgG antibodies in the EPS

Patient	Antibodies in EPS		Antibodies in serum		*C. trachomatis* antigen			Leukocytes in EPS (in the visual field)
	IgA	IgG	IgM	IgG	IgA	urethra	EPS	
1	(+)	(+++)	(–)	(++)	(+)	(–)	(–)	> 10
2	(+)	(–)	(+)	(+)	(+)	(+)	(+)	> 10
3	(+)	(–)	(+)	(+++)	(–)	(–)	(–)	> 20
4	(+)	(+++)	(–)	(+++)	(+)	(–)	(–)	> 10
5	(+/–)	(–)	(+)	(+)	(–)	(–)	(–)	(–)
6	(+)	(+)	(–)	(+)	(–)	(–)	(–)	> 15
7	(+)	(+++)	(–)	(+)	(+)	(–)	(–)	> 15
8	(+)	(+++)	(–)	(++)	(+)	(–)	(–)	> 20
9	(+)	(+)	(–)	(+)	(+)	(–)	(–)	> 10
10	(+)	(–)	(–)	(+)	(+)	(+)	(–)	> 10
11	(+)	(+++)	(–)	(+++)	(+)	(–)	(+)	cover visual field
12	(+)	(–)	(–)	(+)	(–)	(–)	(–)	> 20
13	(+)	(+++)	(+)	(+++)	(+)	(–)	(+)	> 15

(+) titers 1:50–1:400, (++) titers 1:1600–1:6400, (+++) titers > 1:12800

of collecting material. As a first step, smears were taken from the urethra, then, after micturition by the patient, the prostate gland was massaged and the secretion obtained tested. A similar method of collecting samples was applied by Bruce and Reid[2], Goh et al.[5], and Kobayashi et al.[8], who obtained positive direct test results only in the EPS.

In chlamydial infection diagnosis, the problem of serological blood tests is still a controversial one. The detection of specific antibodies is of particular importance in persons with deep organic changes, such as pelvic inflammatory diseases, prostatitis, or epididymitis, where antigen detection is difficult[16]. Recently, attention has been drawn to the presence in patients with chronic infections and complications of atypical forms of *Chlamydia*, with changed antigen properties, different from the elementary and retinal bodies, which do not multiply but remain alive and are potent immunogens stimulating the production of specific antibodies[1, 16]. The methods of detecting the *C. trachomatis* antigens and the culture method may not reveal altered forms of the bacteria, thus giving falsely negative results.

In the serum of the patients studied, IgG antibodies were the most frequently detected (50%), in contrast to IgM and IgA, which were twice as rare (IgM 19.4%, IgA 25%). In all the men studied, the titers of IgM and IgA in the serum were low and interpreted as weakly positive. On the other hand, IgG antibodies were found to have a high titer in 2/3 of the cases. Similar or higher percentages in the frequency of IgG antibody detection in the serum of chronic prostatitis patients were noted by Grant et al.[6] (100%), Peeters et al.[20] (49%), and Weidner et al.[27] (73%), but lower percentages were given by Kojima et al.[10] (7.5%), Miyata et al.[17] (29%), and Tekgül et al.[23] (35.5%). In the literature there are only a few reports on the detection of specific IgM and IgA antibodies in the serum, and the results indicate that they are rarely present in the male population with chronic prostatitis[3, 14, 15]. In our material a correlation between the presence of *C. trachomatis* infection of the prostate and the presence of specific serum antibodies, mainly IgA and IgG, more rarely IgM, was observed. Similar observations were made by Tekgül et al.[23]

In recent years some reports have appeared concerning the role of serological tests of the semen[9] or EPS[11, 25] in the diagnostics of prostatitis, with emphasis being laid on the non-invasive character of this type of test and the ease with which the material is obtained, as in the detection of specific antibodies in the serum.

In the study presented here, specific IgA antibodies were detected in the EPS in 33.3% and IgG in 36.1% of the patients. In the EPS, antichlamydial antibodies occurred three times more frequently than the EPS *C. trachomatis* antigen. It should be noted that the titers of IgA antibodies detected in the EPS were much higher than those of the IgG antibodies, which is the reverse of the values noted in the serum. There was a high correlation between the presence of IgA (83.3%) and IgG (100%) antibodies in the EPS and that of IgG antibodies in the serum. Of the 3 patients with chlamydial infection of the prostate, IgA antibodies were found in the EPS of two and IgG in all three.

In the literature to date there have been few reports concerning serological studies of the EPS. Shortliffe

et al.[22], in determining specific IgA antibodies in the EPS, detected them in 20% of the NBP (non-bacterial prostatitis) patients and in 12% of the control males. However, they did not observe any correlation between the presence of anti-*C. trachomatis* IgA antibodies in the EPS and the presence of specific IgG antibodies in the serum.

Tsunekawa et al.[24], like the present authors, found that IgA and IgG antichlamydial antibodies occurred far more frequently in both the EPS and serum of chronic prostatitis patients than in the control group. These authors noted the presence of IgA antibodies with a high titer and IgG with a low titer in the EPS and the reverse in the serum[24, 25]. These observations are in agreement with those made in our study. The high values of the specific antibodies of IgA detected in the EPS indicate a local stimulation of the immunological response by the *Chlamydia* in the prostate which, in turn, suggests the possible induction of inflammation of the gland by these microorganisms. Other Japanese authors detected antibodies of IgA only in the EPS of chronic prostatitis patients; the percentages were similar to those obtained in our study: 31.5%[15] and 29%[11]. Koroku et al.[11] noted a high correlation between the presence of anti-*C. trachomatis* IgA antibodies and the intensification of the leukocyte reaction in the EPS. In none of the literature available has a comparison of the results of serological tests and direct bacteriological tests of the prostate secretions for *C. trachomatis* infection been found.

In conclusion, a high level of anti-*C. trachomatis* IgA antibodies and a low level of IgG in the EPS, in contrast with the serum, indicates an active local inflammatory process of the prostate induced by *Chlamydia*. Serological tests of the EPS are a useful additional method in the diagnosis of chronic prostatitis.

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