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# The effect of *Chlamydia trachomatis* infection of the prostate gland on the concentration of citric acid

**Bożena Zdrodowska-Stefanow[1], Iwona Ostaszewska-Puchalska[2], Jerzy Badyda[3] and Zofia Galewska[4]**

[1] Department of Dermatology and Venerology, Medical University, Białystok, Poland
[2] Center for Sexual Transmitted Disease Research and Diagnostics, Białystok, Poland
[3] Outpatients Urology Clinic "Multimedica", Białystok, Poland
[4] Department of Medical Biochemistry, Medical University, Białystok, Poland

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## Abstract

**Introduction:** The objective of the study was to assess the relationship between *Chlamydia trachomatis (C.t.)* infection of the prostate and the concentration of citric acid.
**Materials and Methods:** The study involved 60 patients with chronic prostatitis (NIH III). Urethral swabs and expressed prostatic secretions (EPS) were collected for analysis. The urethral swabs were tested for PMNs and the presence of *Neisseria gonorrhoeae* and *C.t.*, while the EPS were analyzed to determine PMN count, *C.t.*, and citric acid concentration. The DFA or LCR method was used for *C.t.* diagnosis. The concentration of citric acid was measured using the UV method.
**Results:** Inflammation of the prostate (PMNs ≥10/field) was diagnosed in 58.3% of the patients. *C.t.* infection was found in 20%, including 8.3% with only the urethra affected and 10% with only the prostate. One patient had both the urethra and the prostate infected. A reduction in the concentration of citric acid in EPS was observed in 56.7% of the men. In 88.2% of the patients, reduced citric acid concentration was accompanied by an elevated PMN count in the EPS. All patients with *C.t.* infection of the prostate showed a reduced concentration of citric acid. In five patients with urethral infection, lack of a decrease in this parameter was noted in one. In all the patients with chlamydial infection, irrespective of localization, a high PMN count was observed in the EPS.
**Conclusions:** Determination of the concentration of citric acid in the prostatic fluid is a good indicator of prostatitis. *C.t.* infection of the prostate gland is accompanied by a decrease in the concentration of citric acid.

**Key words:** *Chlamydia trachomatis*, citric acid, polymorphonuclear leukocyte, urethra, prostate gland, expressed prostatic secretions.

**Corresponding author:** Iwona Ostaszewska-Puchalska, Center for Sexually Transmitted Disease Research and Diagnostics, Św. Rocha 3, 15-879 Białystok, Poland, tel.: +1 85 74-60-454, fax: +1 85 74-36-30, e-mail: centstd@poczta.onet.pl

## INTRODUCTION

Prostatitis is the most common disorder among men under 50 years of age who report to the urological outpatient departments [1]. Half of all men experience symptoms of prostatitis at some time in their lives [19]. One of the causes of this ailment is infection by *Chlamydia trachomatis* (*C. trachomatis*), a sexually transmitted intracellular microorganism [10, 22]. Chlamydial infection of the prostate gland is frequently asymptomatic or oligosymptomatic and may therefore persist for many years [7]. An elevated polymorphonuclear leukocyte (PMN) count in the expressed prostatic secretions (EPS) is the major marker used for prostatitis evaluation [21]. Another parameter of normal prostate gland function which is altered in prostatitis is the concentration of citric acid [9, 11]. The most substantial amounts of citric acid are produced in the prostate gland, which is also its greatest reservoir [9].

The objective of the study was to evaluate the concentration of citric acid in patients with chronic prostatitis and to determine the relationship between this concentration and *C. trachomatis* prostatic infection.

## MATERIALS AND METHODS

The study involved 60 patients aged 16–73 years (mean: 40 years) with chronic prostatitis referred to the Center for Sexually Transmitted Disease Research and

Diagnostics in Białystok from urological outpatient departments. These patients belonged to group III according to the National Institutes of Health (NIH) prostatitis classification of 1995, which is chronic prostatitis/chronic pelvic pain syndrome (CP/CPPS) [15]. A detailed history of the course, character, and duration of the disease and its treatment was elicited from the patients and then the material was collected for analysis. None of the patients had received antibiotics for at least 3 months preceding the study.

The method of Meares and Stamey was used for diagnostics [18]. Only patients with negative leukocyturia and bacteriuria as well as with excluded urinary bladder inflammation, based on urinalysis and bacteriological tests (from middle stream), were included in the study. First, urethral swabs for *Neisseria gonorrhoeae* (*N. gonorrhoeae*), *C. trachomatis*, and for the presence of PMNs were obtained after at least 2 h following the last micturition. Then the patients in whom urethritis and *N. gonorrhoeae* infection were excluded underwent prostatic massage following micturition. The prostatic fluid flowing from the urethra was collected and evaluated for *C. trachomatis* and other pathogens (Gram-negative and Gram-positive organisms, *Mycoplasma hominis, Ureaplasma urealyticum*, *Candida* sp., *Trichomonas vaginalis*), PMN count, and concentration of citric acid. Further studies involved only those patients whose EPS tests were negative for pathogenic microorganisms or in whom there was chlamydial infection.

Direct preparations were made on microscopic slides from the urethral swabs using sterile plastic loops and from drops of prostatic fluid. After fixing and staining by Gram's method, the smears were evaluated for the presence of *N. gonorrhoeae* and leukocytes were counted. Urethritis was diagnosed at ≥4 and prostatitis at ≥10 PMNs per field of vision under a light microscope at 1000×.

The culture method using PVX media (BioMerieux, France) was applied to diagnose *N. gonorrhoeae* infections. For the diagnostics of mycoplasmal infection, Mycoplasma IST 2 strip was used (BioMerieux, France) and for the detection of Gram-negative and Gram-positive bacteria, *T. vaginalis*, and *Candida* sp., routine culture methods were used. *C. trachomatis* infection in urethral smears was detected by means of direct immunofluorescence assay (MicroTrak, Syva, USA) and in EPS by means of the ligase chain reaction method (LCx, Abbott, USA) [16].

The concentration of citric acid was measured at the Department of Medical Biochemistry, Medical University of Białystok, using the ultraviolet method (TC Citric Acid, Boehringer, Germany) [25]. The normal concentration range is 18.84±0.72 mg/ml (18.12–19.59 mg/ml) [8]. Values of 12.01–18.11 mg/ml were treated as a small decrease, 7.01–12.0 mg/ml as a moderate decrease, and ≥7.0 mg/ml as a high decrease.

This study was approved by the university's ethics committee.

## RESULTS

Based on the prostatic PMN count evaluation, inflammation of the prostate was diagnosed in 35/60 (58.3%) patients involved in the study (NIH IIIa – inflammatory CP/CPPS). In 25/60 (41.7%) men an elevated leukocyte count in the EPS was not detected (NIH IIIb – noninflammatory CP/CPPS; Fig. 1). Table 1 presents the PMN counts in the EPS in a group of 35 men with diagnosed NIH IIIa prostatitis. The most common count was ≥10 PMNs in a field of vision (40%); other counts were ≥15 PMNs (31.4%), ≥20 PMNs (17.1%), and ≥30 PMNs (8.6%). In one patient, leukocytes covered the whole field of vision.

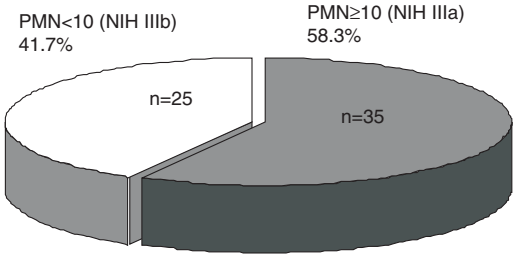


**Fig. 1.** Classification of patients with chronic prostatitis (NIH III) regarding the presence of the elevated PMN count (≥10 per field) in EPS (n=60)

**Table 1.** Leukocyte counts in EPS in group of 35 men with diagnosed inflammation of the prostate (PMNs ≥10 per field)

| Leukocyte count in EPS per field of vision | Patients with inflammation of the prostate (N=35) | |
|---|---|---|
| | n | % |
| ≥10 | 14 | 40.0 |
| ≥15 | 11 | 31.4 |
| ≥20 | 6 | 17.1 |
| ≥30 | 3 | 8.6 |
| Cover field of vision | 1 | 2.9 |
| Total | 35 | 100 |

*C. trachomatis* infection was found in 12/60 (20%) men (Fig. 2), including 5/60 (8.3%) only in the urethra and 6/60 (10%) only in the prostate. One patient had

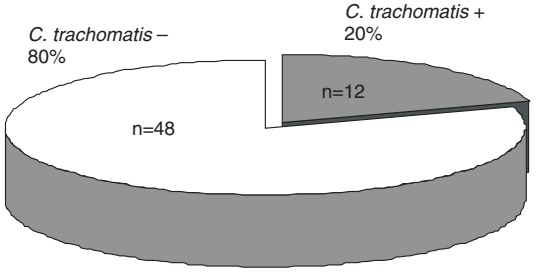


**Fig. 2.** Classification of patients with chronic prostatitis (NIH III) regarding the presence of *C. trachomatis* infection in urethra or/and in EPS (n=60)

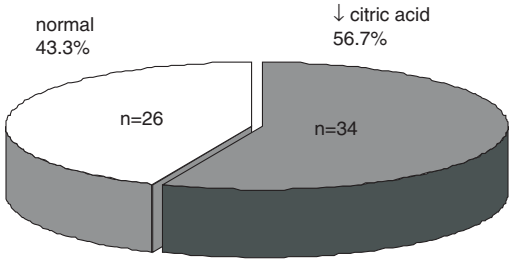


**Fig. 3.** Classification of patients with chronic prostatitis (NIH III) regarding the value of the citric acid concentration in EPS (n=60)

both the urethra and the prostate affected. Twelve cases of chlamydial infection were detected in the group of 35 patients with leukocyte count ≥10 per field in the EPS (34.3%), while none of the 25 patients without elevated PMN counts had *C. trachomatis* infection.

A reduction in the concentration of citric acid in the prostatic fluid was observed in 34/60 (56.7%) men with chronic prostatitis (CP/CPPS; Fig. 3), with a small decrease being the most common (22/34 patients – 64.7%). Moderate and high decreases were more rare, each found in 6/34 (17.6%) men. In most of the patients (30/34 – 88.2%) the reduced citric acid concentration was accompanied by an elevated PMN count in the EPS. In the other 26 patients of the group studied a reduction in citric acid concentration was not observed, and in only 5 (19.2%) cases was an elevated leukocyte count ascertained. Figure 4 presents the concomitant occurrence of citric acid decrease and prostatitis. Elevated leukocyte count in the EPS was found to accompany a high decrease in citric acid concentration. The highest decrease in the concentration of citric acid was observed in men with ≥30 PMNs per field (2.15 mg/ml and 4.98 mg/ml) and in the patient with leukocytes covering the whole field of vision (3.97 mg/ml). In the three remaining men who presented with a high decrease in citric acid concentration, the PMN count in EPS was ≥15 per field.

Figure 5 presents the results of PMN analysis and the concentration of citric acid in the EPS in patients with diagnosed *C. trachomatis* infection. All chlamydial patients, irrespective of the infection site, had elevated PMN counts in the EPS. The seven patients with *C. trachomatis* infection of the prostate gland showed a reduced concentration of citric acid in the EPS, the decrease being high in four men (57.1%). In the group of five patients with urethral infection, one showed no decrease in the concentration of citric acid in the prostatic fluid, and in the remaining four the decrease was small or moderate. A high decrease in the concentration of citric acid most frequently coexisted with chlamydial infection of the prostate gland (4/7 patients – 66.7%). In the case of urethral infection alone, the decrease was usually small (3/5 patients – 60%).

## DISCUSSION

The PMN count is considered the best known marker of inflammation in the genitourinary tract [21] and is a routine diagnostic parameter. Other inflammation markers include PMN elastase, citric acid (a prostate gland function parameter), fructose (a seminal vesicle function indicator), and α-glucosidase (a marker of epididymal function) [2]. The prostate gland is the major site of citric acid production and its greatest reservoir in the body [3]. The production is regulated by testosterone and prolactin [4, 6]. The greatest amounts of citric acid are produced by epithelial cells of the peripheral part of the gland, where also its concentration is the highest [14]. Literature data point out the high prognostic value of citric acid concentration in inflammatory

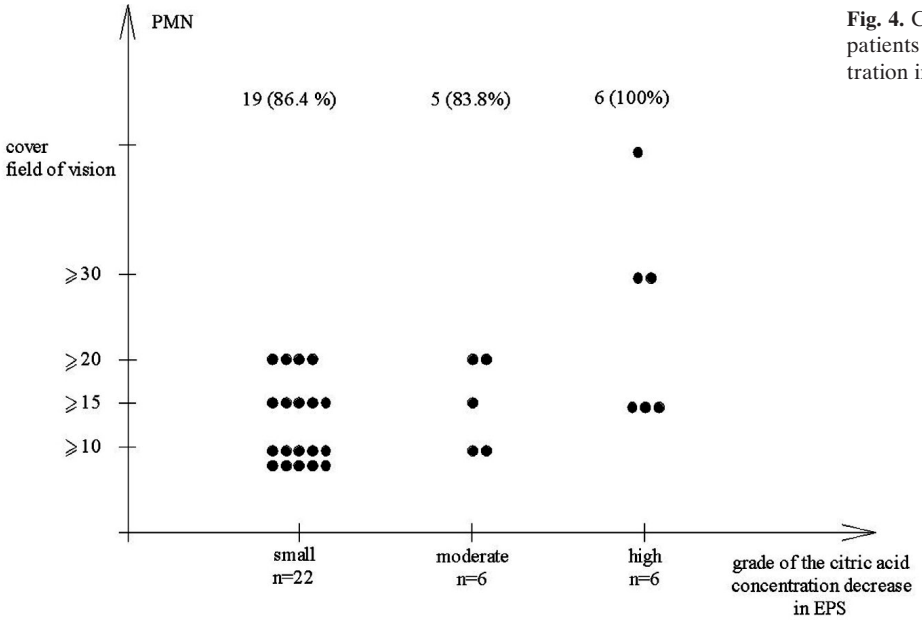


**Fig. 4.** Coexistence of elevated PMN count in patients with the decreased citric acid concentration in EPS (n=34)

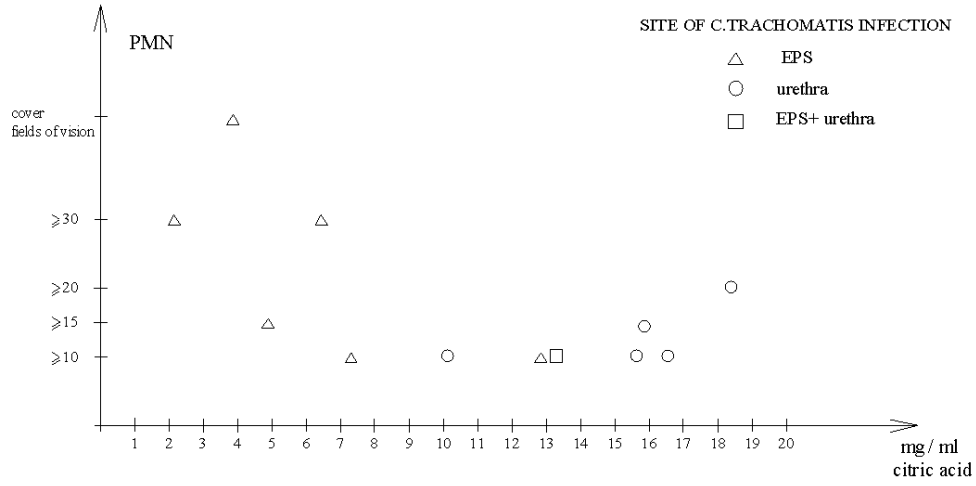


**Fig. 5.** PMN test results and citric acid concentration in EPS of 12 patients with diagnosed *C. trachomatis* infection.

states and prostate carcinoma, which are characterized by its drop [2, 4, 5, 11]. Decreases in the concentration of citric acid are especially high (even 20-fold) in patients with prostate carcinoma and can thus be used to facilitate differentiation between carcinoma and benign hypertrophy, in which citric acid secretion is either lacking or increases [5, 12, 13]. According to literature, the concentration of citric acid can be determined in the prostatic fluid [8, 17] and in semen [2, 20]; however, different norms have to be applied to each determination. A decrease in the concentration of citric acid, irrespective of the study material, always indicates a prostate gland dysfunction.

In the current study on a group of men with chronic prostatitis, we found a reduction in the concentration of citric acid in the EPS in 56.7% of cases. The decreases were extremely high in patients with high leukocyte counts in the prostatic fluid and in patients with *C. trachomatis* infection of the prostate gland. All the patients with diagnosed prostatic chlamydial infection demonstrated a decrease in citric acid concentration. There are only single literature reports on the relationship between *C. trachomatis* infection of the male genitourinary organ and decreased citric acid concentration. Wolff et al. [24] noted a significant decrease in the concentration of citric acid in the semen of infertile men who were *C. trachomatis* positive. In another study, Wolff [23] detected low concentrations of citric acid in infertile men whose semen had high PMN counts. This indicated the coexistence of asymptomatic prostatitis, responsible for PMNs in the semen. Van der Kammer et al. [20] determined the concentration of citric acid in the semen of men with chronic prostatitis, acute epididymitis, and inflammation of other adnexitis, and examined urethral swabs for *C. trachomatis* infection. They found a reduced concentration of citric acid in all the study groups of men, without any significant differences. A difference was evident in comparison with controls. No literature data have been available on studies concerning *C. trachomatis* infection of the prostate gland in combination with the concentration of citric acid in the prostatic fluid.

In conclusions, determination of the concentration of citric acid in the prostatic fluid is a good indicator of prostatitis. *C. trachomatis* infection of the prostate gland is accompanied by a decrease, usually high, in the concentration of citric acid.

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