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Incidence of Chlamydial Uterine Cervix Infections in South-West Poland in the Period of 1996–2004

Występowanie zakażeń szyjki macicy wywołanych przez chlamydie w południowo-zachodniej Polsce w latach 1996–2004

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Abstract

Objectives. Assessment of the incidence of uterine cervix infection caused by *Chlamydia trachomatis* in south-west Poland in the period of 1996–2004.
Material and Methods. Two methods used for the laboratory diagnosis of the disease were compared. The specificity and sensitivity of the ligase chain reaction (LCR) method versus the direct immunofluorescence (DIF) test were evaluated. The material was cervical smears from 320 patients with different genito-urinary tract diseases. The patients were being treated in gynecological hospital units and outpatient departments (n = 255), private gynecological clinics (n = 45), the Wrocław Health Care Center (n = 12), special-service and police hospital (n = 6), an infectious and invasive diseases hospital (n = 1), and the Tuberculosis Care Center (n = 1). The smears were tested simultaneously by LCR (*Chlamydia trachomatis* assay, Abbott) and DIF (Chlamyset, Orion Diagnostica), applying monoclonal antibodies labeled with isothiocyanin fluoresceine.
Results. In 1996–1999, the LCR sensitivity in comparison with DIF was 83.3% and specificity 99.6%, whereas the DIF sensitivity in comparison with LCR was 71.4% and specificity 100%. In 2000–2004, positive results were achieved in 109/401 (27.1%) patients. Increasing percentages of infected women were observed in the consecutive years: 21.2%, 25.5%, 28.5%, 29.7%, 31.5%.
Conclusions. The incidence of chlamydial uterine cervix infections in south-west Poland in the period of 1996–2004 is approximately seven times lower than that observed in 1986. The investigations performed in 2000–2004 revealed the presence of *Chlamydia trachomatis* in 1/3 of women with urogenital tract infections. This situation requires appropriate treatment to avoid remote serious complications (**Adv Clin Exp Med 2006, 15, 3, 427–433**).

Key words: *Chlamydia trachomatis*, uterine cervix infections, ligase chain reaction method, direct immunofluorescence test.

Streszczenie

Cel pracy. Ocena częstości występowania zakażeń szyjki macicy o etiologii *Chlamydia trachomatis* w Polsce południowo-zachodniej w latach 1996–2004.
Materiał i metody. Porównano dwie metody badań stosowanych w diagnostyce tego schorzenia. Oceniono swoistość i czułość reakcji łańcuchowej ligazy (LCR) oraz immunofluorescencji bezpośredniej. Materiałem do badań w latach 1996–1999 były rozmazy z kanału szyjki macicy pobrane od 320 pacjentek, a w latach 2000–2004 od 401 pacjentek z różnymi schorzeniami układu moczowo-płciowego. Pacjentki leczono na szpitalnych oddziałach ginekologicznych oraz w przychodniach (n = 255), prywatnych klinikach ginekologicznych (n = 45), we Wrocławskim Centrum Zdrowia (n = 12), szpitalu MSWiA (n = 6) oraz w szpitalu chorób zakaźnych (n = 1), szpitalu gruźliczym (n = 1). Próbki badano jednocześnie techniką LCR (test *Chlamydia trachomatis*, firmy Abbott) oraz testem immunofluorescencji bezpośredniej DIF (Chlamyset, Orion Diagnostica), wykorzystującym przeciwciała monoklonalne znakowane izotiocyjanianem fluoresceiny.
Wyniki. W latach 1996–1999 czułość metody LCR w porównaniu z DIF wynosiła 83,3%, swoistość – 99,6%, a czułość DIF w porównaniu z LCR wynosiła 71,4%, swoistość – 100%. W latach 2000–2004 wyniki dodatnie uzyskano u 109/401 (27,1%) pacjentek. Procentowy wzrost liczby zakażonych kobiet obserwowany w kolejnych latach wynosił: 21,2; 25,5; 28,5; 29,7 oraz 31,5%.

Wnioski. Częstość występowania chlamydialnych zakażeń szyjki macicy w południowo-zachodniej Polsce w latach 1996–2004 jest około 7 razy niższa od obserwowanej w 1986 r. W badaniach prowadzonych w latach 2000–2004 stwierdzono obecność *Chlamydia trachomatis* u 1/3 kobiet z zakażeniami układu moczowo-płciowego. W celu uniknięcia poważnych komplikacji w tych schorzeniach należy zastosować odpowiednie leczenie (**Adv Clin Exp Med 2006, 15, 3, 427–433**).

Słowa kluczowe: *Chlamydia trachomatis*, LCR, DIF, zakażenia szyjki macicy.

Chlamydia trachomatis is considered to be an important cause of pelvic inflammatory disease (PID) and its sequelae, such as infertility, chronic pelvic pain, and ectopic pregnancy [1]. Recent studies showed a reduction in PID incidence connected with the screening and proper treatment of cervical chlamydial infections [2].

The uterine cervix is susceptible to various sexually transmitted infections, including *Chlamydia trachomatis*. In most infected women, this induces very few, if any, clinical symptoms. Undiagnosed and untreated infections allow the microorganisms to ascend the female genital tract, causing acute inflammation and its sequelae. A number of comparative studies with cell cultures as well as antigen detection methods have found nucleic acid amplification of cervical, urethral, and vulvar swabs samples to be highly accurate in diagnosing of lower female genital tract infections [3, 4].

Some authors have shown that the presence of chlamydiae in cervical smear samples in patients with clinical, laparoscopical, or histological evidence of PID does not correlate well with female upper genital tract infections [5]. Laparoscopy, used as a gold standard in the diagnosis of PID, does not seem to be 100% accurate in direct visualization of upper tract inflammatory diseases. Moreover, transvaginal sonography, a non-invasive procedure performed in the doctor's office, is considered to be observer-dependent. Magnetic resonance imaging is a new technique which usefulness for such diagnosis has not yet been evaluated in prospective clinical trials [6, 7].

The aim of the study was to assess the incidence of uterine cervix infections caused by *Chlamydia trachomatis* using laboratory methods. Two of these methods were also compared with respect to sensitivity and specificity.

Material and Methods

Cervical smears were tested to detect *Chlamydia trachomatis* presence. The analyzed material (n = 320) came from patients suffering from different gynecological diseases in the period of 1996–1999. They were hospitalized in the gynecological departments of two university and two municipal hospitals (n = 257), treated in municipal as well as regional outpatient departments (n = 18), and in private outpatient clinics (n = 45).

Two cervical swabs were taken from each patient included into the study by a specialist during gynecological examination. Using the original transport sets, the samples were transferred to the Chlamydia Diagnostics Scientific Laboratories at the Department of Microbiology of Silesian Piasts University of Medicine in Wrocław. One of the swabs was tested using LCR and the second using Chlamyset. The patients' ages ranged from 18 to 75 years.

The following methods of chlamydial infection diagnostics were used:

1) Ligase Chain Reaction (LCR) (*Chlamydia trachomatis* assay, Abbott) preceded by appropriate preparation of the sample tested. The samples were heated in a dry water bath at 97ºC for 15 minutes, then cooled to room temperature for 15 minutes. Simultaneously, the calibrator and the negative control were prepared by the addition of 0.1 ml of activator and centrifuged until the reagent reached 2/3 of the volume. Then, 0.1 ml of the cooled sample as well as N-control and C-calibrator in two repetitions were added to each amplification test tube. The tubes were then tightly closed and placed in the thermocycler. After two hours of amplification, the test tubes were centrifuged again for 15 seconds and then placed in a determined sequence in appropriate reaction cells of the whirling arm. The charged whirling arm was placed into the reaction chamber of the LCx analyzer. At the same time, the open box containing thoroughly mixed reagents was inserted into the heating jacket of the analyzer. The test results were obtained after 60 minutes. The result was evaluated as positive when the ratio of the examined value to the cutoff value was > 1.

2) Direct Immunofluorescence (DIF) (Chlamyset test, Orion Diagnostica) applying monoclonal antibodies labeled with isothiocyanin fluoresceine. The test procedure and interpretation of the results were performed according to the reagents manufacturer's recommendations [3].

In the period of 2000–2004, cervical swabs were taken from 401 women (age range: 21–60) as material for *Chlamydia trachomatis* detection. In this group of patients only the Direct Immunofluorescence (DIF) method (MicroTrak Chlamydia Direct Specimen Test, Trinity biotech, Ireland)

was used. The technique is based on detecting of elementary chlamydial bodies, which shine as a result of specific monoclonal antibody binding.

Results

The evaluation of the sensitivities and specificities of LCR versus DIF and DIF versus LCR (n = 320) in cervical smear analysis are presented in Table 1. Compared with DIF, LCR sensitivity was 83.3% and specificity 99.6%, whereas DIF sensitivity was 71.4% and specificity 100% compared with the LCR method.

The clinical data of the 320 examined patients, as reported by their physicians, are shown in Table 2. These patients were included in the laboratory detection of *Chlamydia trachomatis* using LCR.

In this group, chlamydial infection was diagnosed in 7 patients (2.1%): 2 with cervicitis, 2 with uterine cervix erosion, and 1 with colpitis, adnexitis, and sterility.

The data concerning the ages of the examined women in whom the presence of Chlamydia trachomatis in the cervical smears was detected are illustrated in Table 3.

The highest percentage of *Chlamydia trachomatis* infection (4.5%) was observed in the group of women 18–27 years old. In the group of women aged 28–37 years, this microorganism was detected in only one patient. In the remaining age groups, no *Chlamydia trachomatis* was found.

In the population of the region of Lower Silesia, *Chlamydia trachomatis* was found in 2.2% of patients from private outpatient clinics, 2.0% of patients hospitalized at the Second Department of Gynaecology, and 1.7% at the First Department of Gynecology and Obstetrics of Silesian Piasts University of Medicine in Wrocław. In the vaginal smears obtained from the patients treated in the five remaining centers, no *Chlamydia trachomatis* presence was detected, as shown in Table 4.

Table 1. Sensitivity and specificity of LCR and DIF tests

Tabela 1. Czułość i swoistość metod LCR i DIF

		DIF	
		+	–
LCR	+	5	1
	–	1	313

Sensitivity – 5/6 (83.3%)
Specificity – 313/314 (99.6%)

		LCR	
		+	–
DIF	+	5	0
	–	2	313

Sensitivity – 5/7 (71.4%)
Specificity – 313/313 (100%)

Table 2. Clinical data of women with positive *Chlamydia trachomatis* LCR test results

Tabela 2. Dane kliniczne kobiet z pozytywnym wynikiem testu LCR w kierunku *Chlamydia trachomatis*

Clinical data (Dane kliniczne)	No. of tested (Liczba badanych)	Positive results (Wyniki pozytywne)	
		n	%
Infertility	98	1	(1.0)
Vaginitis*	48	1	(2.0)
Cervicitis	38	2	(5.2)
Vaginal discharge	30	0	–
Cervical erosion	25	2	(8.0)
Appendicitis	23	1	(4.3)
Adnexal tumor	14	0	–
Ovarian cyst	10	0	–
P.C.O.S.	8	0	–
Cervical cancer	8	0	–
Ectopic pregnancy	6	0	–
Turner syndrome	3	0	–
Hypogastric pain	4	0	–
Habitual abortion	3	0	–
Pre-in vitro examination	2	0	–
Total (Suma)	320	7	2.1

* vaginitis of undetermined etiology.

* zapalenie pochwy o nieustalonej etiologii.

Table 3. Frequency of *Chlamydia trachomatis* infection (%) in women of different age groups (1996–1999)

Tabela 3. Występowanie zakażenia *Chlamydia trachomatis* u kobiet w zależności od grupy wiekowej (1996–1999)

Age of patients (Wiek pacjentek)		18–27	28–37	38–47	48–57	58–67	68–75
No. of tested (Liczba badanych)		133	130	45	9	2	1
Positive results (Wyniki pozytywne)	n	6	1	0	0	0	0
	%	(4.5)	(0.76)				

Table 4. Frequency of *Chlamydia trachomatis* infection in women from different out- and in-patient departments

Tabela 4. Występowanie zakażenia *Chlamydia trachomatis* u kobiet leczonych ambulatoryjnie i na różnych oddziałach szpitalnych

Patients (Pacjentki)	No. of tested (Liczba badanych)	Positive results (Wyniki pozytywne)
		n (%)
II Department of Gynecology	199	4 (2.0)
I Department of Gynecology and Obstetrics	56	2 (3.5)
Private gynecological clinics	45	1 (2.2)
Wroclaw Health Care Centers*	8	0
Special-Service and Police Hospital	6	0
South-west Poland regional Health Care Centers**	4	0
Infectious and Invasive Diseases Hospital	1	0
Tuberculosis Care Center	1	0
Total (Suma)	320	7 (2.1)

* ZOZ Krzyki (n = 4), ZOZ Żelazna (n = 2), ZOZ Psie Pole (n = 1), ZOZ Fabryczna (n = 1).

** ZOZ Oleśnica (n = 2), ZOZ Kąty Wrocławskie (n = 1), ZOZ Legnica (n = 1).

Table 5. Cervical smear test results for *Chlamydia trachomatis* using DIF in the years 2000–2004

Tabela 5. Wyniki badania rozmazu szyjki macicy w kierunku *Chlamydia trachomatis* z użyciem metody DIF w latach 2000–2004

Year (Rok)	No. of tested (Liczba badanych)	Positive results (Wyniki pozytywne)	
		n	%
2000	85	18	21.1
2001	86	22	25.5
2002	70	20	28.5
2003	84	25	29.7
2004	76	24	31.5
Total (Razem)	401	109	27.1

In the period of 2000–2004, positive results were obtained in 109/401 (27.1%) patients. Increasing percentages of infected women in consecutive years were observed: 21.2%, 25.5%, 28.5%, 29.7%, and 31.5% (Table 5). The frequencies of *Chlamydia trachomatis* infection in various groups of patients were compared. Positive chlamydial cervical smears were detected more often in the cases of adnexitis (61.5%), vaginal discharge (36.6%), and miscarriage (31.5%), and less often in cervical erosion (20.0%), cervical cancer (13.7%), and infertility (12.2%). The differences were statistically significant. *Chlamydia trachomatis* was found in 19/66 (28.7%) women with general urogenital tract infections, in 13/48 (27.0%) cases with cervicitis, and in 11/47 (23.4%) patients with vaginitis (Table 6). The highest percentage of chlamydial infections (31.6%) was detected in women ranging in age from 21 to 30 and the lowest (10.0%) in patients aged 51–60 (Table 7). In a control group of 50 women, *Chlamydia trachomatis* was found in 5/50 (10.0%) cases.

Table 6. Clinical data of women with positive *Chlamydia trachomatis* DIF test results in the years 2000–2004

Tabela 6. Dane kliniczne kobiet z pozytywnym wynikiem testu w kierunku *Chlamydia trachomatis* wykonanego metodą DIF w latach 2000–2004

Clinical data (Dane kliniczne)	No. of tested (Liczba badanych)	Positive results (Wyniki pozytywne)	
		n	%
Cervical erosion	65	13	20.0
Cervicitis	48	13	27.0
Vaginitis	47	11	23.4
General urogenital tract infection	66	19	28.7
Vaginal discharge	60	22	36.6
Infertility	41	5	12.5
Adnexitis	26	16	61.5
Cervical cancer	29	4	13.7
Miscarriage	19	6	31.5
Total (Suma)	401	109	27.1

Table 7. Frequency (%) of *Chlamydia trachomatis* infection in women in different age groups (2000–2004)

Tabela 7. Występowanie zakażenia *Chlamydia trachomatis* u kobiet w różnych grupach wiekowych (2000–2004)

Age (Wiek)		21–30	31–40	41–50	51–60
No. of tested (Liczba badanych)		180	127	74	20
Positive results (Wyniki pozytywne)	n	57	32	18	2
	%	31.6	25.1	24.3	10.0

Discussion

The essential material for *Chlamydia trachomatis* detection is cervical smears in women and urethral smears in both men and women [1, 3, 5, 6]. Of the various methods of detecting *Chlamydia trachomatis* presence, two were applied in described investigation: LCR – ligase chain reaction (Chlamydia trachomatis assay) and DIF – direct immunofluorescence (Chlamyset test). DIF has been known and applied in Poland for 13 years for the diagnostic evaluation of urethral as well as cervical smears in patients with urogenital inflammatory diseases. LCR has been available in Poland for 4 years. It is an automatic diagnostic test that, according to the manufacturer's recommendations, may be used to examine urethral and cervical smears as well as urine samples [1, 3, 8–10].

The analysis of the results of comparing the application of LCR and DIF in the diagnosis of *Chlamydia trachomatis* revealed significant correlation of both methods, as presented in a previous report [3].

Göessens et al. [5] evaluated the sensitivity of LCR, Cobas Amplification, and AMT CT in comparison with the culture method based on cervical smear examination results in 456 women. They obtained sensitivities of 84.0%, 93.0%, and 85.0% respectively.

Comparing the same test results with those of PCR, they estimated the LCR, Cobas Amplification, and AMT CT sensitivities as 84.0%, 93.0%, and 85.0%, respectively. The specificity of all three tests was 99.0%.

Schachter et al. [11] performed a comparative trial of the cervical smear analyses of 2132 patients using LCR and the culture method. In comparison with the culture method, LCR sensitivity was 91.4% and specificity 95.8%.

In presented study, of cervical smear examinations in 320 women, *Chlamydia trachomatis* was detected in 2.1% of cases using LCR and DIF. Simultaneously, the evaluation of the sensitivity and the specificity of LCR compared with the DIF method revealed values of 83.3% and 99.6%, respectively. Also, the biographical data and own observations show that during the last four years the lowest percentage of chlamydial infections was detected in the examined group from the region of south-west Poland.

Andrews et al. [1] analyzed the results of cervical smears and urine samples in 462 patients admitted to family planning clinics. They revealed *Chlamydia trachomatis* presence using the LCR and DIF methods in 6.1%, 18.2%, and 16.9% of cases, depending on the method applied and the kind of material evaluated. These authors compared LCR and culture test results in cervical smears for *Chlamydia trachomatis* detection. LCR test sensitivity of 90.3% and specificity of 100% was estimated. Examining urine samples with the same methods they obtained values of 83.9% and 99.5%, respectively.

Bassiri et al. [2] performed a comparative evaluation of LCR, culture, and EIA test results of 447 cervical smears and urine samples. In their study, *Chlamydia trachomatis* infection was found in 3.1% of cases using the LCR method. Moreover, infection was detected using the culture method in 9, EIA test in 3, Plasmid LCR in 14, and MOMP-1cr test in 16 of the women.

The same authors estimated LCR sensitivity and specificity in urine samples analysis as 87.5% and 100%, respectively, and EIA sensitivity and specificity in that material as 18.8% and 100%, respectively. The culture method sensitivity and specificity in the cervical smear examinations was evaluated as 56.3% and 100%, respectively.

Lee at al. [12] performed a comparative trial detecting the *Chlamydia trachomatis* presence in cervical smears as well as in urine samples of 1937 women living in four various geographical regions, i.e. Birmingham (n = 194), Hamilton (n = 240), San Francisco (n = 1086), and Seattle (n = 415). They used the LCR and the culture methods, obtaining positive results in 7.7% and 5.4% of women, respectively.

Comparing the results obtained using LCR versus the culture methods, they found 93.8% sensitivity and 99.9% specificity, and in comparing the culture method versus LCR these values were 65.0% and 100%, respectively.

The discrepancies in the values of LCR specificity reported by different authors may be caused by various factors, e.g. the appropriate smear sampling technique [7, 10, 13–15].

The results of the LCR sensitivity evaluation reveal that methods based on nucleic acid analysis are characterized by higher sensitivity in comparison with culture and LCR methods [3, 4, 8, 9, 16].

Howell et al. [9] evaluated the costs and efficacy of DNA amplification methods based on a *Chlamydia trachomatis* infection screening trial performed in 18,000 women admitted to family planning clinics. They found high sensitivity as well as high specificity of these methods in the urine samples and the cervical smears analysis. These new tests reveal greater possibilities of detecting infection than the commonly used immunoenzymatic assay, but they are more expensive. Despite this, the authors believe that the screening trial costs are lower than those of treating advanced, previously undetected infections.

Both the biographical data and own observations reveal a decrease in the chlamydial infection frequen-

cy in the region of south-west Poland by a factor of seven from that observed in 1986 [3, 17–19].

This may result from the availability of new analytical detection methods with high sensitivity and specificity [20, 21]. It may also be due to widespread prophylaxis education as well as the simultaneous treatment of sexual partners with new-generation antibiotics (macrolides, azylides, tetracyclines, and fluoroquinolones) which are available on the Polish pharmaceutical market [22–24].

The authors conclude that the low percentage (2.1%) of positive results of cervical smears analyses for *Chlamydia trachomatis* testified to the decrease in this microorganism's importance in the etiopathogenesis of the genital infections diagnosed in the region of south-west Poland. The *Chlamydia trachomatis* assay demonstrated a sensitivity similar to that of the Chlamyset test; using these methods, *Chlamydia trachomatis* infection was detected in 2.1% and 1.8% of the examined patients, respectively. The clinical group with the highest percentage of positive LCR test results consisted of women with pelvic pain syndrome and with cervical erosion. *Chlamydia trachomatis* infections were detected most frequently in the cervical smears of sexually active, 18- to 27-year-old women. Cervical testing for *Chlamydia trachomatis* should be performed in all sexually active women between 20 and 30 years of age. The treatment of sexual partners should also be mandatory.

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