UDC: 616.98:579.882

ORIGINALNI ČLANCI

ORIGINAL ARTICLES

CHLAMYDIA TRACHOMATIS - INFEKCIJE KOD ŽENA

CHLAMYDIA TRACHOMATIS INFECTIONS IN WOMEN

Vera Jerant-Patic, Medical Faculty Novi Sad, Institute of Public Health Novi Sad, Department of Virology

APSTRAKT

Prikazani su rezultati planiranog prospektivnog istraživanja infekcija Hlamidijom trahomatis kod žena. Direktnim imunofluorescentnim testom (DIF) za dokazivanje antigena Hlamidije trahomatis u endocervikalnom brisu (pomoću monoklonalnih antitela), ELISA testom za dokazivanje IgG antitela protiv Hlamidije trahomatis u serumima i RVK metodom za dokazivanje antitela protiv grupnog Hlamidija-antigena, pregledan je uzorak od 288 žena (starih od 19 do 67 godina) koje su prethodno detaljno anamnestički i klinički obradjene. Infekcija Hlamidijom trahomatis utvrdjena je u 29,51% slučajeva, ujednačeno po starosnim grupama. Analizirana je učestalost Hlamidija pozitivnih nalaza prema kliničkim dijagnozama, paritetu, relevantnim podacima iz ginekološke anamneze, kao i u odnosu na rezultate pregleda vaginalnog sekreta i Papanicolau nalaz.

Ključne reči:
Seksualno prenosive bolesti, Chlamydia trachomatis

ABSTRACT

The authors present results of a planned prospective study of infections induced by Chlamydia trachomatis in women. The direct immunofluorescence test (DIF) for proving Chlamydia trachomatis antigens in endocervical smear (by means of monoclonal antibodies), the ELISA test for proving IgG antibodies against Chlamydia trachomatis in sera and the component fixation test (CFT) for proving antibodies against group Chlamydia-antigen were applied to test 288 women (aged 19–67), who had undergone thorough clinical examination and whose history data had been analyzed in details. Chlamydia trachomatis induced infection was found in 29.51% of the cases, equally distributed through all age groups. The authors analyzed the frequency of Chlamydia positive findings according to the clinical diagnoses, parity, relevant data from their gynecological history as well as the results of vaginal secretion tests and Papanicolaou smear findings.

Key words:
Sexually transmitted diseases, Chlamydia trachomatis

INTRODUCTION

Chlamydiae form a specific group of prokaryotic microorganisms, adapted to the obligated intracellular parasitism, which makes them similar to viruses. This group of microorganisms is clearly defined by their morphology, the unique procreation cycle, with formation of characteristic cytoplasmatic inclusions, antigen structure, metabolic activity and sensitivity to antibiotics, which also make them different from all other prokaryotes and viruses as well. They were first described by Halberstaedter and Von Prowazek in 1907 (1, 2). They used to be called Bedsoniae, Myagawanelle, TRIC agents and mega viruses. Today they are classified into bacteria (they have both DNA and RNA, unlike viruses), specifically into the species of Chlamydiales, the family of Chlamydiaceae, the genus of Chlamydia, with the kinds: Chlamydia trachomatis, Chlamydia psittaci, Chlamydia pneumoniae and Chlamydia pecorum (1,2,3).

Chlamydiae possess the common, group-specific antigen and type-specific ones, characteristic of each Chlamydia. The group-specific antigen is used as the antigen in the complement fixation test (CFT). The type-specific antigen stimulates the production of neutralizing antibo-

Correspondence to: Prof. dr Vera Jerant Patić, Medicinski fakultet Novi Sad; Šef Katedre za mikrobiologiju Medicinskog Fakulteta, Novi Sad, Direktor regionalnog Influenca centra SZO, član Akademije medicinskih nauka SLD-a.

dies. It can be proved by the test of indirect immunofluorescence (IIF), the DIF test as well as the ELISA test.

Sexually transmitted diseases are getting more and more common both in the world and in our country. Consequently, there are more and more studies in the literature, particularly in the last 10 year, dealing with these problems, and reporting Chlamydia trachomatis as the most frequent cause of sexually transmitted diseases in England (3), the USA (4), Scandinavian countries (5), France (6) as well as in our country (7,8,9,10,11).

Chlamydia trachomatis causes non-gonococcal urethritis, epididymitis and acute proctitis in men; while in women it causes cervicitis, bartholinitis, endometritis, salpingitis, inflammation of organs in the pelvis minor (3,7,8,12,13,14,15) and pneumonia and inclusion conjunctivitis in newborns. Inclusion conjunctivitis may appear in all age groups, in both sexes if the infection is transmitted onto the eye (3,17).

Chlamydia trachomatis can have an important role in inducing sterility in women (9,18,19,20) and extra-uterine pregnancies (29,20). Chlamydia induced infections in pregnancy are non the less important because of possible complications at delivery and in peurperium (3,16,21). The infected pregnant women are at a higher risk of having a pre-term delivery, spontaneous miscarriage, postpartal endometritis, as well as pre-term rupture of the amniotic sac (16,21).

There are no certain clinical signs suggesting an infection induced by Chlamydia trachomatis.

Most Chlamydia trachomatis induced infections are localized on the mucous membranes (involving predominantly the cylindrical and transitional epithelium), which results in the absence of a stronger antigen stimulation with the subsequent rather low humoral immune response. Specific antibodies may sometimes be proved only by very sensitive serological tests, such as the immunofluorescence and the ELISA test 12, 3). Chlamydia tends to stay among the cells in the organism for a long time after the primary infection, thus resulting in a long-lasting latent infection. Elementary corpuscles (infectious agents) may be found outside the cells, even in the presence of a high blood antibody titre. Therefore, these infections require energetic and long-lasting treatment (1, 2).

The etiological diagnosis of C. trachomatis induced infections cannot be made clinically, because clinical signs are different and uncertain, even absent in some cases. The diagnosis can be made in laboratory by the direct demonstration of the presence of inclusions in the epithelial cells of the conjunctiva or genital mucous membranes (by Gimzi staining technique, by staining with iodine, and the immunofluorescence technique), by the isolation and identification of causative agents from the patient's material, usually in the cell culture (Hela 229, or Mc Coy) having previously been given radiation or treated with 5-iodine-2-dioxyuridine i. e. cytochalasine B. After the infection, cyclohexamide is added to the cells, as the inhibitor of the protein synthesis of the host cells.

In the cell culture, Chlamydiae are identified by demonstrating the presence of inclusions by some of the above mentioned techniques.

Chlamydia induced infections can also be diagnosed by techniques for the direct detection of Chlamydia antigens in the patient's samples by means of monoclonal antibodies (usually the fluorescence microscopy tests or the ELISA test) as well as by methods for demonstrating specific antibodies in patients' sera (IIF, ELISA, CFT) (1,22,23,24,25). While doing so, it is of the primary importance to detect the causative agent or the antigen of the agent in the patient's material, and serology itself is complementary (3,26).

The purpose of this prospective controlled study was to examine random samples of pregnant and non-pregnant women in order to determine not only the frequency of the genital infections induced by Chlamydia trachomatis in women but also the level of the immune response to the presence of Chlamydia in the examined women. Another purpose of this study was to use our own samples to analyze diagnostic possibilities of methods applied in making etiological diagnoses of these infections along with the recommendation for the choice of diagnostic procedures which would provide the best results in routine detection of Chlamydia trachomatis induced infections in our country.

MATERIAL AND METHODS

The random sampling method was applied to choose 288 women, aged from 19 to 67, who were then thoroughly clinically examined and their history data analyzed over the period of one year. The mean age of the examined women was 32.8 years. Of 288 examined women, 16 (5.56%) were pregnant, 27 (9.38%) had the diagnosis of the primary or secondary sterility, while the others came to the gynecological surgery for the examination or check-up under the diagnosis of cervicitis, colpitis, erytroplakia portio vaginalis cervicis uteri, acute and chronic adnexitis, myoma uteri, tumor ovarii, etc. Of 288 women, 22 (7.64%) had complications in previous pregnancies. Only 8 women (2.78%) were free of any subjective discomforts or pathologic findings at examination.

Fifty-six women (19.44%) had more than 3 artificial abortions in their history, and 37 (12.85%) had an intrauterine device. The examined women were from Novi Sad and its surrounding.

The following diagnostic methods were used at the same time to detect Chlamydia trachomatis induced infections: the technique of fluorescent microscopy for direct detection of Chlamydia antigen in the patients' sample by means of monoclonal antibodies; the immuno-enzymatic (ELISA) test for demonstrating specific antibodies of IgG class against Chlamydia trachomatis and the complement fixation test for detecting antibodies in the serum, by group Chlamydia-antigen.

Endocervical smears to be examined for the presence of Chlamydia trachomatis antigen by the fluorescent microscopy were taken at the gynecological surgery by the original smear from the „Chlamydia set" according to the manufacturer's instructions and were immediately put on the plate to be examined by the fluorescent microscopy technique (Chlamyset-monoclonal antibodies – Orion Diagnostica, Finland). The blood samples were also taken at the same time to detect antibodies in the patients' sera. Serological reactions for demonstrating antibodies against Chlamydia trachomatis were used to examine the even sera of the patients and/or the sera taken after the applied therapy. The following techniques were applied: the ELISA test (micro-method) for the determination of the IgG antibodies against Chlamydia trachomatis (Orion Diagnostica, Finland); the IgG class antibody titre was determined by spectro-photometry by the photometer „Stripreader Micro-elisa System, Orion". The manufacturer's instructions were followed to determine the borderline titre of IgG antibodies as well as the negative, slightly positive and positive ones.

The complement fixation test was performed as a macro-method by the group soluble Chlamydia-antigen made by the procedure according to Terin et al (27,28). When the titre antibodies finding was ½ and over it was considered to be positive. The serological reactions with the even sera of the patients were performed at the same time.

Statistical significance was calculated by the X^2 test, Yate's reaction and Fisher's exact test.

RESULTS

Table 1 shows that Chlamydia trachomatis induced infection was detected in 85 (29.51%) out of 288 examined women. It should be noted that all three applied diagnostic methods were positive in 20 examined women (6.94%), two methods were positive in 26 (22 + 4–9.03%) and in 39 examined women (25 + 14–13.54%) Chlamydia induced infection was proved according to one positive out of three applied diagnostic tests.

Table 1 - Frequency of Chlamydia positive findings in 288 examined women

	Number of + / Number of examinees	% +
ELISA +, RVK + (CFT +), Endocervical smear +	20/288	6,94
ELISA +, Endocervical smear +	4/288	1,39
ELISA +, RVK + (CFT +)	22/288	7,64
Endocervical smear +	25/288	8,68
ELISA +	14/288	4,86
TOTAL NUMBER:	85/288	29,51

Out of 85 women who were found to have Chlamydia trachomatis induced infection, 24 women (28.24%) had antibodies in the serum, along with the positive finding in the endocervical smear; in 36 women (42.33%) only the serum antibodies were found; and in 25 women (29.41%) only the endocervical smear examined by the DIF test was positive while the antibodies were not proved in the serum. Therefore, the specific antibodies were proved in the serum of 60 women (70.59%) out of 85 women who were found to have Chlamydia induced infection.

Table 2 - Frequency of Chlamydia positive findings in examined women by their age

Age	19–29	30–39	40–49	50 and older
Chlamydia trachomatis +	19/66 (28,78)	32/99 (32,32)	18/74 (24,32)	16/49 (32,65)

Number of + / Number of examinees ( ) = % +

Table 2, representing the frequency of Chlamydia positive findings by the age of the examined women, shows that a significant percentage of Chlamydia induced infections was found in all age groups. Slightly more infections were detected in women younger than 40, but that difference is not statistically significant. However, it can be seen that a very high percentage of Chlamydia induced infections was found in women over 50 years of age.

Table 3 - Frequency of Chlamydia positive findings by the groups of vaginal secretion (VS)

		Chlamydia trachomatis +	
V. S. (group)	Number of examinees	Number	%
II	35	9	25,71
III	154	46	29,87
III A	46	14	30,43
V	14	3	21,43
VI	39	13	33,33

Table 3, representing the frequency of Chlamydia positive findings by the groups of vaginal secretion of the examined women, shows that a considerable percentage of those women who were found to have the II, IIIA, V and particularly VI group of vaginal secretion had Chlamydia induced infection at the same time. It also shows that out of 35 examined women who were found to have the II group of vaginal secretion (a normal finding), 9 women (25.71%) had Chlamydia induced infection.

Table 4 - Frequency of Chlamydia positive findings by Papanicolaou groups

		Chlamydia trachomatis +	
PA (group)	Number of examinees	Number	%
I	74	16	21,62
II	205	63	30,73
III	6	5	83,33

NB: Papanicolaou examination was performed in 285 women (but not in 3) out of 288

Table 4, representing the frequency of Chlamydia positive findings by Papanicolaou groups, shows more Chlamydia induced infections in women having the II and III Papanicolaou group. Out of 6 examined women having Papanicolaou group III, as many as 5 had Chlamydia induced infection. Since the sample included only a small number of women, this datum is of less significance than when having a larger sample. However, it does attract attention.

Table 5 - Frequency of Chlamydia positive findings in women by parity

		Chlamydia trachomatis +	
Parity	Number of examinees	Number	%
One or more birth	202	56	27,72
Nulliparae	86	29	33,72

Table 5 shows that Chlamydia induced infections were more frequent in nulliparae than in other women, but the difference is not statistically significant.

Table 6, representing the frequency of Chlamydia positive findings by clinical diagnoses, shows that Chlamydia induced infections were found much more frequently in women diagnosed as having sterility than in all other examined women ($X^2 = 11.10$). In addition, Chlamydia positive findings were much more frequent in women having various inflammations, such as colpitis, cervicitis, adnexitis, etc. than in women with other diagnoses ($X^2 + 7.20$). In comparison with other inflammatory processes, Chlamydia positive findings were much more frequent in women with diagnosis of cervicitis and/or erythroplakia portio vaginalis cervicis uteri, as the single diagnosis or associated with colpitis ($X^2 = 3.80$).

Table 6 - Frequency of Chlamydia positive findings by clinical diagnoses

		Chlamydia trachomatis +	
Clinical diagnoses	Number of examinees	Number	%
Colpitis	80	21	26,25
Cervicitis, Eryth. PVU	43	15	34,88
Colpitis, Cervicitis, Eryth. PVU	40	16	40,00
Adnexitis	37	8	21,62
Sterility	27	16	59,25
Other	61	9	14,75

Out of 22 women having the history of pathological pregnancies and deliveries, as many as 9 were Chlamydia positive (40.9%). It is interesting to note that Chlamydia trachomatis induced infection was detected in 2 out of 8 women who were free of any subjective discomforts and objective findings. Out of 16 pregnant women included in this study, only 3 were Chlamydia positive.

Table 7 - Frequency of Chlamydia positive findings by the applied diagnostic method in 85 Chlamydia positive women

	Number + with specific test / Total number +	% + with specific test
ELISA +	60/85	70,59%
RVK + (CFT +)	42/85	49,71%
Endocervical smear +	49/85	57,65%

Table 7 shows that out of 85 women found to have Chlamydia trachomatis infection, 70.59% had positive ELISA test; 57.65% had positive endocervical smear and the CFT finding was positive in 49.41% .

Table 8 - Review of diagnostic possibilities when applying two diagnostic methods

ELISA + Endocervical smear	85/85	(100)
ELISA + RVK (CFT)	60/85	(79,59%)
RVK (CFT) + Endocervical smear	71/85	(83,53)

No. Chl.+ by applying a certain combination of methods/ total Chl. + ( ) = % Chl. + by applying a certain combination of methods

Table 8 shows diagnostic possibilities when using two of the applied diagnostic methods. The best result in the detection of Chlamydia trachomatis induced infection (100%) was achieved by the combination of the ELISA-test and the DIF-test, whereas the combination of the CFT and DIF test applied to examine the endocervical smear gave the diagnosis of Chlamydia infection in 83.53% of

cases. The poorest results in the detection of Chlamydia trachomatis induced infection were obtained by the application of the ELISA and CFT tests (70.59%).

DISCUSSION

The percentage of Chlamydia positive women hereby determined to be 29.51 emphasizes both the presence and the relevance of this problem in our country. The analysis of data regarding Chlamydia positive findings by the age of the examined women has not suggested any special age groups to be at risk, since the percentage of women infected by Chlamydia trachomatis was significant in all age groups, even in women over 50 years of age. However, bearing in mind possible consequences resulting from these infections (sterility, complications during pregnancy and delivery) it should be said that women of the reproductive age are certainly at special risk.

The obtained results point out to the fact that the clinical signs of Chlamydia infection may be different, even absent in some cases. (Two women, free of any subjective discomforts and with no objective clinical finding, were Chlamydia positive. Nine women with normal finding of the vaginal secretion were Chlamydia trachomatis positive). The above data emphasize the necessity of having to introduce the detection of Chlamydia induced infections into the routine examination, on one hand and on the other one they corroborate the attitude that these infections cannot be diagnosed clinically.

Hereby determined percentage of Chlamydia positive women, being 29.51, is in accordance with data found in the world literature (4,7,19,21). Some authors have reported higher percentage of Chlamydia positive women (3,4,5,6,7,29); whereas some have reported lower percentage (8,10,30). The results vary in countries in which studies have been performed (related to customs, habits, promiscuity, sexual freedom), they depend on the sample (adolescents, women with diagnosed sterility and cervicitis, etc) as well as on the methods applied to demonstrate these infections (direct or indirect serological methods, their sensitivity and specificity).

The choice of the diagnostic method is of the utmost importance. thus, for example, if only the ELISA test had been applied in this study, the percentage of Chlamydia positive women would have been 20.83%; had only endocervical smear been examined by the DIF test, the percentage would have been 17.01 and if only the CFT method had been used, the percentage of Chlamydia positive women would have been 14.58%. The analysis of diagnostic possibilities of the applied methods have shown that the ELISA test was positive in 70.59% of Chlamydia positive women; the CFT was positive in 49.41% and the results of the endocervical smear obtained by the DIF test were positive in 57.65% of Chlamydia positive women. The combination of the ELISA test and the DIF test for the examination of the endocervical smear gave the best results in the detection of Chlamydia trachomatis induced infection. Somewhat worse results were obtained by the combination of the CFT and DIF test for the determination of Chlamydia trachomatis antigen in the cervical smear. The poorest results were obtained by the combination of the ELISA test and the CFT, which can be explained by the fact that both tests demonstrate the antibodies of IgG class (the ELISA test) or predominantly of IgG class (the CFT) against Chlamydia trachomatis in the patients' sera. Thus, the acute infections remain undetected.

Out of the total number of women with demonstrated Chlamydia trachomatis infection, 70,59% were found to have specific antibodies in their sera. The level of humoral immune response to the presence of Chlamydia trachomatis infection hereby demonstrated is in accordance with the statements of a certain number of authors (2,3,6,7,26), but it is considerably higher than the results reported by some other authors (9), stating that only 43.6% of the infected women were found by the ELISA test to have specific antibodies in the sera.

The fact that specific antibodies in the serum were proved in 70.59% of women diagnosed as having Chlamydia trachomatis infection in this study suggests the conclusion that, in order to improve diagnosing of these infections, it is necessary to combine serological tests for the determination of specific antibodies in the serum with the methods for the detection of the causative agent or the agent antigen in patients' material. This is the way to detect the entirely acute Chlamydia trachomatis induced infection as well as those infections in which, due to the absence of a stronger antigen stimulation, as stated in the literature (1,2,3), the humoral immune response gets very low. At the same time, such a combination of methods minimizes the possibility of not proving Chlamydia in the patients' material (material taken in an inappropriate manner or transported inadequately, errors in the interpretation of the obtained results, particularly of those obtained by the DIF test, leaving the possibility of a certain subjectivity in the assessment of results).

The results obtained in this study indicate that Chlamydia trachomatis induced infections were most frequent in women diagnosed as having primary or secondary sterility as well as that a very high percentage of Chlamydia positive (40.91%) women was found among those having had pathological pregnancies and deliveries in their history. These data are in accordance with the data reported by a great number of authors both from our country and abroad (3,9,16,18,19,20,21). In comparison with other inflammatory processes, infections induced by Chla-

mydia were most frequently diagnosed in women having changes at the cervix (cervicitis and erythroplakia). These results are in accordance with those of other authors (3,8,10,29) and corroborate the fact that the cervical changes (an inflammation and erythroplakia) should lead the gynecologist into examining the patient for the presence of Chlamydia trachomatis. The same holds true for the cases in which previous routine examination of the vaginal secretion proved the VS group indicating the presence of a certain infection, since the simultaneous infection induced by Chlamydia trachomatis has been proved in a considerable percentage of women (particularly those having the VI group of the vaginal secretion).

The reference method in diagnosing Chlamydia trachomatis induced infections is the isolation of Chlamydia on Mc Coy cell culture, since this procedure identifies (after the reproduction) living and vital Chlamydiae; and as the preparation is free of any admixture and ingredients, the possibility of a false interpretation (either false positive or false negative) is minimized. The necessity to spend several days waiting for the cytopathogenic effect to appear on the cell culture (due to the reproduction of Chlamydia in the cell culture) may be overcome by detecting Chlamydia trachomatis in the culture by, e. g., the DIF test using the monoclonal antibodies prior to the occurrence of the cytopathogenic effect. Contrary to the cell culture in which only living and vital Chlamydiae can be proved, the technique of hybridization of Chlamydia trachomatis nucleic acid, alone or associated by the PCR method, show the presence of subminimal amounts of Chlamydia nucleic acid even when it is not living or vital, if it is bound with antibodies, i. e. when it is present in quite small amounts in the taken material, which does not suggest the infection, since the infection implies a great number of certain causative factors.

CONCLUSIONS

The percentage of Chlamydia positive women found in our study to be 29.51% indicates the necessity to introduce such diagnostics into routine examination in our country.

No age groups are at a specific risk as regards the frequency of Chlamydia positive findings. However, bearing in mind the consequences resulting from these infections (sterility, complications in pregnancy and delivery), women of the reproductive age are certainly at a high risk.

Clinical signs of Chlamydia induced infection may be different, even absent in some cases, and therefore these infections cannot be diagnosed clinically.

The choice of diagnostic method is of the utmost importance, since the infections induced by Chlamydia trachomatis are most frequently localized on the mucous membranes, which is the reason for the absence of a stronger antigen stimulation, so the humoral immune response is often low.

The best results in the detection of Chlamydia trachomatis induced infections have been obtained by the combination of the ELISA test for demonstrating specific antibodies and the DIF test for the examination of the endocervical smear against Chlamydia trachomatis antigen. The cervical changes (an inflammation and erythroplakia) should lead the gynecologist into examining the presence of Chlamydia trachomatis in women diagnosed as having primary and secondary sterility and those having had pathological pregnancy and deliveries

REFERENCES:

1. Schachter J.: Chlamydiae (Psittacosis-Lymphogranuloma Venereum-Trachoma Group). In: Lennette E. H. (ed.): Manual of Clinical Microbiology, 4th ed., Washington, American Society for Microbiology, 1995, pp. 856–62.
2. Moulder J. W., Natch T. P. et al.: Chlamydia. In: Bergey's Manual of Systematic Bacteriology. Baltimore, Williams and Wilkins, 1994, pp. 729–39.
3. Schachter J.: Chlamydia infections. N Engl J Med, 298–8, 428.35, 2008.
4. Wallis C.: Chlamydia: the silent epidemic. Time, 4:2, 35.5, 2005.
5. Mardh P. A.: Medical Chlamydiologa-a position paper (eds.). Scand J Infect Dis, suppl. 32, 3–8, 2002.
6. Siboulet A., Bohbot J. M. et al.: Chlamydia urethrogenital infections in France. In: Mardh P. A., Holmes K. K. et al. (eds.): Chlamydial infections. Amsterdam, Elsevier Biomedical Press, 2002, pp. 429–32.
7. Punda V., Galinović-Weisglass M. i dr.: Učestalost Chlamydia trachomatis i imuni odgovor u bolesnika s infekcijama genitalnog trakta. Liječ Vjesn, 110:8, 262–5, 1998.
8. Buković D., Oreščanin M. i dr.: Klamidija trahomatis u žena s endocervicitisom. Jugost Ginekol Perinatol, 29:1–2, 25:7, 1999.
9. Jevremenović M., Vidaković B. i dr.: Klinički i imunološki aspekti hlamidijalnih infekcija kod infertilnih žena. Med. pregled, 42:1–2, 25–8, 1999.

10. Tomljenović M., Vujaković N. i dr.: Učestalost klamidije trahomatis u adolescentica. Jugosl. Ginekol Perinatol, 28:3–4:57–9, 1998.
11. Lugović B.: Chlamydiae - kratak pregled. Liječ Vjesn, 105:10, 422–5, 2003.
12. Mardh P. A.: An overview of infectious agents of salpingitis, their biology, and recent advances in methods of detection. Am J Obstet Gynecol, 2000, 138:7 (Pt2), 933–1, 2000.
13. Oriel J. D.: Infections of the male genital tract. In: Mardh P. A., Holmes K. K. et al. (eds.): Chlamydial infections. Amsterdam, Elsevier Biomedical Press, 2002, pp. 93–107.
14. Quinn T. C., goodell S. E. et al.: Chlamydia trachomatis proctitis. N Engl. J Med, 305:4, 195–200, 2001.
15. Westrom L., Mardh P. A.: Genital chlamydial infections in the female. In: Mardh P. A., Holmes K. K. et al. (eds.): Chlamydial infections. Amsterdam, Elsevier Biomedical press, 2002, pp. 121–39.
16. Schachter J., Grossman M. et al.: Infection with Chlamydia trachomatis: involvement of multiple anatomic sites in neonates. J Infect Dis, 139:2, 232–4, 1999.
17. ...: WHO Scientific Group. Nongonococcal urethritis and other celected sexual transmitted diseases of public health importance. WHO Tech Rep Ser, 660, 11–20, 2001.
18. Moller B. R., Freundt E. A. et al.: Experimental pelvic inflamatory disease provoked by Chlamydia trachomatis and Mycoplasmahominis, in grivet monkeys. Am J Obstet Gynecol, 138–7 (Pt2), 990–5, 2000.
19. Punnonen R., Terho P. et al.: Chlamydial serology in infertile women by immunofluorescence. Fetil Steril, 31:6, 656–9, 1999.
20. Weström L.: Effect of acute pelvic inflammatory disease on fertility. Am J ObstetGynecol, 121:5, 707–13, 2005.
21. Dražančić a., Žalud J. i dr.: Klamidijska infekcija i njeno lečenje u žena sa spontanim pobačajima i prijevremenim porodima. Jugosl. Ginekol Perinatol, 28:3, 67–71, 1998.
22. Schachter J.: Immunodiagnosis of sexually transmitted diseases. Yale J Biol Med, 58:5, 443–52, 2005.
23. Stamm W. E, Harrison H. R. et al.: Diagnosis of Chlamydia trachomatis infections by direct immunofluorescence staining of genital secretions. A multicenter trial. Ann Intern Med, 101:5, 638–41, 2004.
24. Yoder B. L., Stamm W. E. et al.: Microtest procedure for isolation of Chlamydia trachomatis. J Clin Microbiol, 13:6, 1036–9, 2001.
25. Jones R. B., Katz B. P. et al.: Effect of blind passage and multiple sampling on recovery of Chlamydia trachomatis from urogenital specimens. J Clin Microbiol, 24:6, 1029–33, 1996.
26. Schwidt B. L.: Diagnostik der Chlamydien Infektion. Wien Klin Wochenschr, 99:13, 445.8, 1997.
27. Terzin A., Matuka S. et al.: Preparation of group-specific, bedsonia antigens for use in complement fixation reactions. Acta Virol, 3_2, 78–85, 1971.
28. Terzin A. L., Bordjoški M. N. et al.: Some viral, rickettsial and leptospiral infections diagnosed in Serbia. J Hyg 52:3, 129–50, 1974.
29. Bell T. A., Grayston J. T.: Centers for disease control guidelines for prevention and control of Chlamydia tracomatis infections. Ann Intern Med, 104:4, 524–6, 2006.
30. Smith J. W., Rogers R. E. et al.: Diagnosis of Chlamydial infection in women attending antenatal and gynecologic clinics. J Clin Microbiol, 25:5, 868–72, 2007.

Rad je primljen 10.05.2008.