ORIGINAL PAPERS

Adv Clin Exp Med 2010, **19**, 4, 437–441
ISSN 1230-025X

Magdalena Frej-Mądrzak, Agnieszka Jama-Kmiecik, Irena Choroszy-Król

The Diagnostic Importance of Detecting *Chlamydia Trachomatis* Antigen and Anti-IgG Anti-cHSP60 Antibodies in Infertile Women

Znaczenie diagnostyczne wykrywania antygenu *Chlamydia trachomatis* i przeciwciał IgG anty cHSP60 u niepłodnych kobiet

Department of Basic Sciences, Faculty of Health Sciences, Wroclaw Medical University, Poland

Abstract

Background. Clinical forms of chlamydia infection in women are inflammation of the urethra, urethral syndrome, cervicitis, inflammation of the rectum, and conjunctivitis. Persistence of *Chlamydia trachomatis* infection leads to an increased production of the 60-kDa chlamydial heat-shock protein (cHSP60).
Objectives. The aim of this study was to assess the diagnostic value and usefulness of the cHSP60-IgG-ELISA test and to determine the prevalence of IgG anti-cHSP60 antibodies in infertile women according to age groups.
Material and Methods. The study group consisted of 108 women with primary (n = 91; 84.3%) and secondary (n = 17; 15.7%) infertility. The control group (n = 37) consisted of women without any complaints of the urogenital tract. *C. trachomatis* antigen was determined in cervical smears by the direct immunofluorescence Pathfinder® Chlamydia trachomatis Direct Specimen test from Bio-Rad. IgG anti-cHSP60 antibodies were determined in the serum using the cHSP60-IgG-ELISA test from medac.
Results. The 108 cervical swabs of the women being treated due to infertility were labeled by direct immunofluorescence (DIF); a positive test result was noted in 41 (37.9%) patients. Serum from the 108 was assessed by ELISA and IgG anti-cHSP60 antibodies were found in 17 (15.7%). In the control group, positive results of the *C. trachomatis* test were found in 18.9% of the women by DIF and IgG anti-cHSP60 antibodies were found in the serum of 5 (15.6%).
Conclusions. Serological tests may only supplement bacteriological tests and have some value in epidemiological studies. However, they should not be used as an alternative to bacteriological examination (**Adv Clin Exp Med 2010, 19, 4, 437–441**).

Key words: *Chlamydia trachomatis*, IgG anti cHSP60.

Streszczenie

Wprowadzenie. Kliniczne postacie zakażeń *Chlamydia trachomatis* u kobiet to zapalenie cewki moczowej i zespół cewkowy, zapalenie szyjki macicy, odbytu oraz zapalenie spojówek. Przetrwałe zakażenie chlamydią skutkuje wzrostem wydzielania cHSP60 (chlamydialnego białka szoku termicznego o masie 60 kDa).
Cel pracy. Ocena wartości i przydatności diagnostycznej testu cHSP60-IgG-ELISA oraz określenie występowania przeciwciał IgG anty cHSP60 u niepłodnych kobiet z uwzględnieniem grup wiekowych.
Materiał i metody. Grupę badaną stanowiło 108 kobiet, u których zdiagnozowano niepłodność pierwotną (n = 91; 84,3%) lub niepłodność wtórną (n = 17; 15,7%). Grupę kontrolną stanowiły kobiety (n = 37) bez dolegliwości ze strony układu moczowo-płciowego, które zgłaszały się do poradni ginekologicznej w celu wykonania rutynowych badań profilaktycznych. Do badania antygenów *C. trachomatis* w wymazach z cewki moczowej i szyjki macicy metodą immunofluorescencji bezpośredniej (DIF – *direct immunofluorescence*) zastosowano test Pathfinder® Chlamydia trachomatis Direct Specimen, firmy Biorad. Do wykrywania przeciwciał w surowicy krwi badanych zastosowano test cHSP60-IgG-ELISA firmy medac.
Wyniki. Antygen *Chlamydia trachomatis* wykrywano metodą immunofluorescencji bezpośredniej (DIF) w 108 wymazach z kanału szyjki macicy; wyniki dodatnie uzyskano u 41 (37,9%) kobiet. 108 surowic badano z użyciem techniki ELISA i przeciwciała IgG anty-cHSP60 wykryto u 17 (15,7%). W grupie kontrolnej pozytywne wyniki badań w kierunku *C. trachomatis* uzyskano w 18,9% kobiet techniką DIF, a przeciwciała IgG anty-cHSP60 wykryto w surowicy 5 (15,6%) pacjentek.

Wnioski. Testy serologiczne mogą stanowić uzupełnienie testów bakteriologicznych, mają wartość epidemiologiczną. Nie powinny być jednak alternatywą standardowych badań bakteriologicznych (**Adv Clin Exp Med 2010, 19, 4, 437–441**).

Słowa kluczowe: *Chlamydia trachomatis*, IgG anty cHSP60.

Chlamydia trachomatis is a bacterium with an intracellular development cycle. These microorganisms exhibit tissue tropism and their specific affinity for cylindrical epithelial cells promotes infection within the urogenital tract [1, 2]. As most *C. trachomatis* infections have a mildly symptomatic or asymptomatic course, the bacteria are the most common etiological factor of sexually transmitted diseases. Clinical forms of chlamydia infection in women are inflammation of the urethra, urethral syndrome, cervicitis, inflammation of the rectum, and conjunctivitis [3, 4]. Owing to delayed or unsuccessful treatment, complications may occur such as salpingitis, pelvic inflammatory disease, and ovaritis [5]. It is currently observed that an increase in chronic and recurrent infection results in infertility, miscarriage, or premature birth [6–8]. Persistence of *C. trachomatis* infections leads to an increased production of the 60-kDa chlamydial heat-shock protein (cHSP60). HSP60 is a conservative protein present in both bacteria and humans [9]. The chlamydial amino-acid sequence of the heat-shock protein is 50% homologous to human heat-shock protein hHSP60. This allows immunological cross-reactions which may result in the pathologies of chlamydia infections [10–14].

The aim of this study was to assess the diagnostic value and usefulness of the cHSP60-IgG-ELISA and to determine the prevalence of IgG anti-cHSP60 antibodies in infertile women according to age groups. Correlation of the results and clinical data was assessed.

Material and Methods

The study group consisted of 108 women with primary and secondary infertility who were being treated at the First and Second Departments of Gynecology and Obstetrics, Wroclaw Medical University. The control group (n = 37) consisted of women without any complaints of the urogenital tract. Cervical smears and blood samples were collected from each subject. *C. trachomatis* antigen was determined in the cervical smears by the direct immunofluorescence (DIF) Pathfinder *Chlamydia trachomatis* Direct Specimen test from Bio-Rad. The smear was deemed positive when the sample contained 10 or more elementary bodies of forms of infectious *C. trachomatis*. Anti-IgG anti-cHSP60 antibody was determined in the serum using cHSP60-IgG-ELISA from medac; the result was read spectrophotometrically in the presence of a blank test.

Results

One hundred eight swabs from the cervixes of the women treated due to infertility were labeled by direct immunofluorescence (DIF test) and positive results were noted in 41 (37.9%). The 108 serum samples of these women were assessed by ELISA and IgG anti-cHSP60 antibodies were found in 17 (15.7%). In the 37 women of the control group, positive results of the *C. trachomatis* test were found in 18.9% by DIF and IgG anti-cHSP60 antibody was found in the serum of 5 (15.6%).

There were 91 women with primary infertility (84.3%) and 17 with secondary (15.7%) among the 108 patients. An analysis of the positive results of the clinical data of the patients investigated for *C. trachomatis* by DIF and ELISA showed that the percentage of positive results in the women with primary infertility according to DIF was higher in the assessment of cervical smears (34.1%) than the percentage of *C. trachomatis* infections in the assessment of IgG anti-cHSP60 antibodies in serum by ELISA (18.7%). In the women with secondary infertility, the highest infection rates were found in the examination of cervical smears by DIF (35.3% positive results), whereas there were no positive results when IgG anti-cHSP60 antibodies in serum were tested by ELISA.

In the analysis of the results, differences in the incidence of *C. trachomatis* infection depending on the age of the women were found. The study group was divided into different age groups: 23–27 years (n = 23), 28–32 years (n = 62), 33–37 years (n = 18), and 38–43 years (n = 5). The control group was divided into the same ranges with 11, 19, 3, and 4 subjects, respectively. As it can be seen, the 28–32 age group was the largest in both the study group (57.4%) and the control group (51.4%). The second largest group was the group of women aged 23–27 (21.3% of the study group, 29.7% of the controls). The 33–37 age group there had 16.7% of the women of the study group and 8.1% of the control group. The 38–43 age group had 4.6% of the patients of the study group and 10.8% of the control group. The incidence of *C. trachomatis* infection in the cervical smears and the positive results of IgG

anti-cHSP60 in the blood serum in the different age groups of women of the study group are shown in Table 1 and for the control group in Table 2.

Table 1. The incidence of *C. trachomatis* infection in the cervical smears and the positive results of IgG anti-cHSP60 in the blood serum in the different age groups of women of the study group (n = 108)

Tabela 1. Wyniki dodatnie badań wymazów z szyjki macicy i surowicy krwi w kierunku *Chlamydia trachomatis* w poszczególnych grupach wiekowych, w grupie badanej (n = 108)

Age groups – years (Grupa wiekowa – lata)	Number of samples (Liczba próbek)	The positive results ELISA (Wyniki pozytywne wg ELISA) (%)	The positive results IF (Wyniki pozytywne wg DIF) (%)
23–27	23	5 (21.7)	8 (34.8)
28–31	62	10 (16.1)	23 (37.1)
33–37	18	2 (11.1)	7 (38.9)
38–43	5	0 (0.0)	3 (60.0)

Table 2. The incidence of *C. trachomatis* infection in the cervical smears and the positive results of IgG anti-cHSP60 in the blood serum in the different age groups of women of the control group (n = 37)

Tabela 2. Wyniki dodatnie badań wymazów z szyjki macicy i surowicy krwi w kierunku *Chlamydia trachomatis* w poszczególnych grupach wiekowych, w grupie kontrolnej (n = 37)

Age groups – years (Grupa wiekowa – lata)	Number of samples (Liczba próbek)	The positive results ELISA (Wyniki pozytywne wg ELISA) (%)	The positive results IF (Wyniki pozytywne wg DIF) (%)
23–27	11	2 (18.2)	3 (27.3)
28–31	19	2 (10.5)	3 (15.8)
33–37	3	0 (0.0)	1 (33.3)
38–43	4	1 (25.0)	0 (0.0)

Discussion

The material used for the *C. trachomatis* tests are swabs from the cervix and urethra as well as urine and serum samples. Swabs and urine can be used for the direct detection of the pathogen and in blood serum one can detect different classes of anti-Chlamydia antibodies. Various techniques are also used to detect chlamydia infection, including immunofluorescence, PCR, and ELISA. The test for the determination of IgG anti-cHSP60 antibodies by ELISA is not yet widespread.

Because fertility problems involve a growing group of patients and some of them are caused by complications of *C. trachomatis* infections, the diagnostic tests are being constantly improved. New techniques that can help determine the type of pathogen responsible for infection and the type and degree of infection shortly after the collection of material from the patient may contribute to quicker and more effective clinical diagnosis. Early elimination of *C. trachomatis* allows avoiding many consequences, including obstruction of the fallopian tubes, reduced fertility, or infertility [15]. Many research teams work on such methods and their application in appropriately targeted microbiological diagnostics.

Clad et al. [16], using an enzyme immunoassay technique, examined the serum of 126 women for *C. trachomatis* who had undergone laparoscopy because of infertility, myomas, endometriosis, or pain in the lower abdomen. A positive IgG antibody titer of anti-cHSP60 was found in 49 of the women (38.9%) by ELISA. The authors showed that the presence of IgG anti-cHSP60 antibodies in women with genital infection may indicate an ascending chlamydia infection. In women without clinical signs of infection but in whom the presence of *C. trachomatis* is found, enhanced serological tests should be introduced, which would reduce the growing risk of damage to the fallopian tubes.

In the present study, the presence of IgG anti-cHSP60 antibodies was found in 17 of the 108 infertile women (15.7%) by ELISA, so the rate of chronic infection is lower, as manifested by the occurrence of these antibodies. Karinen et al. [17] analyzed the presence of IgG anti-cHSP60 antibodies in patients aged 31 years among couples with reduced fertility. They found IgG anti-cHSP60 antibodies in 49.6% of the women of the study group and in 41.9% of control women. The authors showed that serological tests were not sufficient evidence to establish a relationship between reduced fertility and the occurrence of IgG anti-cHSP60 antibodies in the serum of the women. However, they did not use DIF for the detection of *C. trachomatis* infection.

In a previous study, the present authors found IgG anti-cHSP60 antibodies at comparable levels in a study (15.7%) and a control group (13.5%), which could confirm the observations of Karinen et al. [17] on the relationship between reduced fertility and the occurrence of IgG antibodies anti-cHSP60 in blood serum. The high percentage of

positive results in the control group indicates that *C. trachomatis* infections are often asymptomatic.

Dutta et al. [18] conducted a comparative study by ELISA and DIF. IgG anti-cHSP60 antibodies were found in patients with secondary infertility who had high rates of reinfection (82.6%) or chronic cervicitis (64.3%). They evaluated the sensitivity of the anti-cHSP60 IgG test by ELISA compared with DIF as 67.3% and specificity as 90.6%. These authors suggested that the detection of IgG anti-cHSP60 antibodies may be useful in the early diagnosis of immunopathological complications in women infected with *C. trachomatis*. They also showed that in women with secondary fallopian tube infertility associated with infection with *C. trachomatis* there is a greater likelihood of IgG anti-cHSP60 antibody occurrence than in women with primary infertility. Among women with secondary infertility, as many as 82% reported chlamydial reinfection, whereas in patients with primary infertility it was usually the first infection. In 2007, Dutta et al. [19] conducted a study of cervical smears of 255 women, 107 of whom had cervicitis and 52 PID. The tests were performed by DIF and ELISA. Chlamydia infection was found in 75 (29.4%) women with cervicitis by DIF and the presence of IgG anti-cHSP60 antibodies was found in 48 (64.0%) of the women with PID by ELISA. A higher percentage of infection was found when examining the levels of specific IgG anti-cHSP60 by ELISA and lower in the *C. trachomatis* antigen test in women with cervicitis.

Analysis of the present results showed that anti-cHSP60 anti-IgG antibody was found in the blood serum of none of the 17 women with secondary infertility, whereas of the 91 women with primary infertility, 17 (18.7%) had positive results.

Chra et al. [20] conducted an analysis of comparative studies on the prevalence of anti-IgG anti-cHSP60 antibodies in the serum of women with or without cicatrized uterine adnexa (peritoneal adhesions) of the pelvis. Of the 33 patients without cicatrized uterine adnexa, IgG anti-cHSP60 antibodies were found in 3 (9.1%). Of the 43 women diagnosed with cicatrized uterine adnexa, IgG anti-cHSP60 antibodies were found in 17 (39.5%). The authors showed that in women with cicatrized uterine adnexa, IgG anti-cHSP60 antibodies occur more frequently and at significantly higher titers. Mascellino et al. [21] conducted similar studies and obtained results that could confirm the above observations. These authors studied a group of 371 women with a diagnosis of PID for anti-cHSP60 antibody in the blood serum. Fully symptomatic pelvic inflammatory disease was diagnosed in 110 patients, whereas in the other 261 women the inflammation was asymptomatic. The analysis of results involved correlation between the prevalence of these antibodies and the course of inflammation (appearance of symptoms) and patient age. In the group of women who had no symptoms of PID, 3.4% had a positive test for anti-cHSP60 antibodies in serum by ELISA. In the patients with fully symptomatic PID, the presence of anti-cHSP60 antibody in blood serum was demonstrated in 20.0% by the same method. Based on the results, the authors stressed the significant association between clinical pelvic inflammatory disease and the occurrence of IgG anti-cHSP60. cHSP60 antibody in the serum of women correlates with the prior infection as well as elevated chlamydial IgA and IgG antibodies in the blood serum. Based on these observations it was found that the use of serological tests to determine the level of chlamydia-specific antibodies may be an important tool in clinical practice which would allow not only early detection of infections, but also avoid the risk of long-term complications of *C. trachomatis* in women.

Cicatrization as a consequence of chronic untreated infection can cause female infertility. A marker of such infections may be IgG anti-cHSP60 antibodies in the blood, and their presence may indirectly indicate an increased risk of fertility problems in infected patients.

The presented review of literature showed that testing for specific IgG anti-cHSP60 antibodies in serum was the subject of many papers. The present study did not confirm the advisability of including this test in the routine diagnostics of infections caused by *C. trachomatis* in women since it has been demonstrated that only in 15.7% of patients with a positive direct immunofluorescence test were IgG anti-cHSP60 antibodies found in serum. Serological tests may only supplement bacteriological tests and have some value in epidemiological studies. However, they should not be used as an alternative to bacteriological examination.

Data from the literature and the present authors' own observations indicating a high prevalence of infections caused by *C. trachomatis* in the urogenital tract in women suggest conducting a comprehensive diagnostics of infections by these pathogens on both the type, frequency of sampling, and selection of appropriate research methods, depending on clinical changes and the nature of the infection.

References

[1] **Zdrodowska-Stefanow B, Ostaszewska I :** *Chlamydia trachomatis* – zakażenia u ludzi. Volumed, Wrocław 2000.

[2] **Kinnunen A:** Chlamydial heat shock protein 60 and cell-mediated immunity in tubal factor infertility. Oulu, Finland 2002.

[3] **Ness RB, Shen C, Bass D, Jackson C, Moysich K, Edwards R, Brunham RC:** *Chlamydia trachomatis* serology in women with and without ovarian cancer. Infect Dis Obstet Gynecol 2008, 20, 1–5.

[4] **Wallin KL, Wiklund F, Luostarinen T, Angström T, Anttila T, Bergman F, Hallmans G, Ikäheimo I, Koskela P, Lehtinen M, Stendahl U, Paavonen J, Dillner J:** A population-based prospective study of *Chlamydia trachomatis* infection and cervical carcinoma. Int J Cancer 2002, 101, 371–374.

[5] **Witkin SS, Linhares IM:** *Chlamydia trachomatis* in subfertile women undergoing uterine instrumentation: an alternative to direct microbial testing or prophylactic antibiotic treatment. Hum Reprod 2002, 17, 1938–1941.

[6] **Idahl A, Boman J, Kumlin U, Olofsson JI:** Demonstration of *Chlamydia trachomatis* IgG antibodies in the male partner of the infertile couple is correlated with a reduced likelihood of achieving pregnancy. Hum Reprod 2004, 19, 1121–1126.

[7] **Wilkowska-Trojmiel M, Zdrodowska-Stefanow B, Ostaszewska-Puchalska I, Redźko S, Przepieść J, Zdrodowski M:** The influence of *Chlamydia trachomatis* infection on the spontaneus abortions. Adv Med Sci 2009, 54, 86-90.

[8] **Witkin SS:** Immunity to heat shock proteins and pregnancy outcome. Infect Dis Obstet Gynecol 1999, 7, 35–38.

[9] **Witkin SS, Askienazy-Elbhar M, Henry-Suchet J, Belaisch-Allart J, Tort-Grumbach J, Sarjdine K:** Circulating antibodies to a conserved epitope of the *Chlamydia trachomatis* 60 kDa heat shock protein (hsp60) in infertile couples and its relationship to antibodies to *C. trachomatis* surface antigens and the *Escherichia coli* and human HSP60. Hum Reprod 1998, 13, 1175–1179.

[10] **Di Felice V, David S, Cappello F, Farina F, Zummo G:** Is chlamydial heat shock protein 60 a risk factor for oncogenesis? Cell Mol Life Sci 2005, 62, 4–9.

[11] **Eggert-Kruse W, Neuer A, Clussmann C, Boit R, Geissler W, Rohr G, Strowitzki T:** Seminal antibodies to human 60kd heat shock protein (HSP 60) in male partners of subfertile couples. Hum Reprod 2002, 17, 726–735.

[12] **Kozakiewicz B:** Nowotwory złośliwe narządu rodnego. Nowa Med Onkol VI 2003, 3 http://www.czytelniamedyczna.pl/nm_on29.php.

[13] **Neuer A, Spandorfer SD, Giraldo P, Dieterle S, Rosenwaks Z, Witkin SS:** The role of heat shock proteins in reproduction. Hum Reprod Update 2000, 6, 149–159.

[14] **Pospisil L, Stroblova H, Canderle J, Unzeitig V:** The chlamydial heat shock protein (cHSP60) in females participating in fertilization programs (a serological study). Acta Dermatoven APA 2004, 13, 75–78.

[15] **Paavonen J, Karunakaran KP, Noguchi Y, Anttila T, Bloigu A, Dillner J, Hallmans G, Hakulinen T, Jellum E, Koskela P, Lehtinen M, Thoresen S, Lam H, Shen C, Brunham RC:** Serum antibody response to the heat shock protein 60 of *Chlamydia trachomatis* in women with developing cervical cancer. Am J Obstet Gynecol 2003, 189, 1287–1292.

[16] **Clad A, Petersen EE, Böttcher M:** Extended *Chlamydia trachomatis* serology: cHSP60 IgG and its association with tubal occlusion. Fifth Meeting of the European Society for Chlamydia Research, Budapest, Hungary, 2004, September 1–4

[17] **Karinen L, Pouta A, Hartikainen AL, Bloigu A, Paldanius M, Leinonen M, Saikku P, Järvelin MR:** Antibodies to *Chlamydia trachomatis* heat shock proteins Hsp60 and Hsp10 and subfertility in general population at age 31. Am J Reprod Immunol 2004, 52, 291–297.

[18] **Dutta R, Jha R, Salhan S, Mittal A:** *Chlamydia trachomatis*-specific heat shock proteins 60 antibodies can serve as prognostic marker in secondary infertile women. Infection 2008, 36, 374–378.

[19] **Dutta R, Jha R, Gupta S, Gupta R, Salhan S, Mittal A:** Seroprevalence of antibodies to conserved region of Chlamydia heat shock proteins 60 and 10 and their possible association with sex hormones in *Chlamydia trachomatis* infected women in India. Br J Biomem Sci 2007, 64, 78–83.

[20] **Crha I, Pospísil L, Stroblová H, Ventruba P, Záková J, Huser M:** Antibodies against the chlamydial heat shock protein in women with periadnexal adhesions. Ceska Gynekol 2006, 71, 127–131.

[21] **Mascellino MT, Ciardi MR, Oliva A, Cecinato F, Hassemer MP, Borgese L:** *Chlamydia trachomatis* detection in a population of asymptomatic and symptomatic women: correlation with the presence of serological markers for this infection. New Microbiol 2008, 31, 249–256.

Address for correspondence:

Magdalena Frej-Mądrzak
Department of Basic Sciences
Wroclaw Medical University
ul. Chałubińskiego 4
50-368 Wrocław
Poland
Tel.: +48 71 784 13 06
E-mail: magdamad@mbio.am.wroc.pl

Conflict of interest: None declared

Received: 28.06.2010
Revised: 23.07.2010
Accepted: 27.07.2010