The chlamydial heat shock protein (cHSP60) in females participating in fertilization programs (a serological study)

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ABSTRACT

Background: Hit shock protein a possible parameter for assessement of chlamidial infections.
Materials: The incidence of IgG antibodies against the chlamydial heat shock protein (cHSP60) and against the species-specific chlamydial major outer membrane protein (MOMP) of *Chlamydia trachomatis (C. t.)* and *Chlamydia pneumoniae (C. p.)* in the blood serum of 70 females attending a fertilization program due to fertility disorders were estimated, and the results compared with those obtained from 50 females suffering from pelvic inflammatory disease (PID) and from 51 female blood donors respectively.
Results: The anti-cHSP60 antibodies have been ascertained as follows: in 26 women from the first group (37.1 %), in 16 of the second group (32.0 %) and in 12 (23.5 %) of the last group. The occurrence of the anti- cHSP60 antibodies in the groups examined was statistically insignificant. Similarly no difference in the occurrence of the species-specific anti-MOMP antibodies *C. t.* and *C. p.* were found in the groups examined.
Conclusion: The occurrence of species-specific antibodies against *C. t*. in women of all groups with positive reaction anti cHSP60 is significantly higher than in those without the antibodies against cHSP60, but against *C. p*. the phenomenon was observed only in the group of the blood donors.

KEY WORDS

chlamydia, heat shock protein, antibodies, fertility disorders, blood donors

Introduction

Practically all cells exposed to sudden changes in the environment respond with an increase in the synthesis of a rather small, heterogeneous group of proteins, varying in size from 10 to 110 kD (1). These proteins were first mentioned in 1962 in connection with the findings of the response to heat attack in the salivary gland chromosomes of the fly *Drosophila busckii*. They were accordingly given the name heat shock proteins (HSPs) - (2). Bacterial cells, similarly to other cells, produce a certain amount of HSP necessary to preserve their vital functions. The shock proteins HSP60 and HSP70 (60 and 70 kD in size, respectively) are immunodominant antigens in a number of microbial pathogens. One of these is *Chlamydia trachomatis*, a microbe that is most often transmitted sexually between people of reproductive age (3, 4).

Recently, we have witnessed the growing interest of clinicians and serologists in this important chlamydial antigen-heat shock protein (cHSP), namely cHSP60. It is generally considered a very important factor in the

Table 1. IgG antibodies against the chlamydial heat shock protein (cHSP 60) in blood sera from women participating in fertilization program, from women suffering from pelvic inflammatory disease (PID) and from female blood donors.

Group of women	*n*	Age (years)[1]	Anti-cHSP 60 positive [n. s.]	Mean index of positivity[1] [n. s.]
Fertilization program	70	30.4 ± 4.4	26 (37.1 %)	3.2 ± 2.0
PID	50	27.5 ± 2.6	16 (32.0 %)	2.8 ± 1.7
Blood donors	51	35.7 ± 10.7	12 (23.5 %)	4.7 ± 3.3

[1] *arithmetic mean ± S.D.*
[n. s.] *The differences between the examined groups are not significant*

Table 2. IgG antibodies against the specific antigens of *C. trachomatis* and *C. pneumoniae* in blood sera from women participating in fertilization programs and from women suffering from PID (patients) in comparison with the results of the same examination female blood donors.

Antibodies anti *C. trachomatis*	Antibodies anti *C. pneumoniae*	Patients (*n* = 120)	Blood donors (*n* = 51)
Positive	Positive	15 (12.5 %)	6 (11.8 %) [n. s.]
Positive	Negative	6 (5.0 %)	3 (5.9 %) [n. s.]
Negative	Positive	54 (45.0 %)	19 (37.3 %) [n. s.]
Negative	Negative	45 (37.5 %)	23 (45.1 %) [n. s.]

[n. s.] *The differences between the examined groups are not significant*

pathogenesis of chlamydial infection (5, 6, 7). Given the fact that the presence cHSP60 can be demonstrated directly (for instance through the use of immunoenzymes) or indirectly, using antibodies developed in the body of a host, it is the aim of this study to indicate the importance of the heat shock protein (60 kD) as an antigen epitope of *C. trachomatis* in cases of fertility disorders, while at the same time making clear the serological influence of antibodies against the analogical protein of *C. pneumoniae* (8, 9, 10, 11).

Examined persons and methods

The incidence of IgG antibodies against the chlamydial heat shock protein (cHSP60) and against the species-specific chlamydial major outer membrane protein (anti-MOMP) of *Chlamydia trachomatis (C. t.)* and *Clamydia pneumoniae (C. p.)* in the blood serum of 70 females attending a fertilization program due to fertility disorders were estimated, and the results were compared with those obtained from 50 women (on average 27.5 years old) suffering from pelvic inflammatory disease (PID) and from 51 female blood donors (on average 35.7 years old).

The antibodies against chlamydial HSP60 (anti-cHSP60) in blood serum were ascertained by a recombinant enzymatic immunoanalysis for the quantitative detection of IgG (anti-cHSP60), using the "cHSP60-IgG-ELISA Medac" device. The species-specific antibodies against the chlamydial major outer membrane protein (anti-MOMP) of *(C. t.)* and *(C. p.)* in the immunoglobulin class of serum IgG were also detected using ELISA diagnostic sets made by Medac (Germany).

The results were evaluated by standard statistical procedures, the chi-squared (χ^2) test for the determination of differences in qualitative data and the Student's *t*-test for the determination of differences in the averages of quantitative data (using Microsoft Excel).

Results

The anti-cHSP60 antibodies were discovered in 26 women from the first group (37.1%), in 16 of the second (PID) group (32.0%), and in 12 of the female blood donors (23.5%). Neither the occurrence of the anti-cHSP60 antibodies nor the average mean index between the groups examined was statistically significant (Table 1).

Similarly, differences in the occurrence of species-specific anti-MOMP antibodies against *C. t.* and *C. p.* were not statistically significant, regardless of the independent or combined evaluation of the two groups of female patients. Specific antibodies against both *C. t.* and *C. p.* were found in 15 (12.5%) of females, antibodies against only *C. t.* in 6 (5.0%) and antibodies against only *C. p.* in 54 (45.0%) of the 120 women in the first two groups. Of the 51 female blood donors, specific antibodies against both *C. t.* and *C. p.* were found in 5 (11.8%) women,

Table 3. IgG - antibodies against the specific antigens of *C. trachomatis* and *C. pneumoniae* resp. in sera of women with a positive or negative reaction to cHSP 60.

Groups Antibodies	Anti- cHSP 60	*n*	Anti-*C. trachomatis*	Anti-*C. pneumoniae*
Fertilization program	Positive	26	8 (30.8 %) *	17 (65.4 %) n. s.
Fertilization program	Negative	44	5 (11.4 %)	20 (45.5 %)
PID	Positive	16	6 (37.5 %) **	12 (75.0 %) n. s.
PID	Negative	34	2 (5.9 %)	19 (55.9 %)
Blood donors	Positive	12	4 (33.3 %) *	9 (75.0 %) *
Blood donors	Negative	39	2 (5.1 %)	15 (38.5 %)

A significant difference of the occurrence of the antibodies against the species-specific chlamydial antigens in the groups positively or negatively anti cHSP 60 reacting persons ($p < 0.05$; ** $p < 0.01$)*

antibodies against only *C. t.* in 3 (5.9%) and antibodies against only *C. p.* in 19 (37.3%) (See Table 2).

C. t. plays an important role in the production of the anti-cHSP60 antibodies in the examined women as is illustrated by the statistically higher occurrence of specific antibodies against *C. t.* in women who were discovered to have anti-cHSP60 antibodies (significantly higher in PID patients) than in those women without anti-cHSP60 antibodies (see Table 3).

Discussion

Infection with *Chlamydia trachomatis* is often a cause of those female fertility disorders that are not - associated with a fallopian tube blockage. The raised sensitivity caused by the chlamydial heat shock protein and the subsequent secretion of the highly homologous human heat shock protein (60 kD) are factors that contribute to the development of fertility disorders from other causes, such as the higher apoptosis of oocytes follicular cells, the production of auto- and isoantibodies against germ cells, etc (11). The very common *C. p.* infection (almost 55% of the women in our study) also in some cases initiates the production of the anti-cHSP60 antibodies because the HSP60 of both species of Chlamydia have identical amino acid sequences in more than 95% of the cases studied.

It is assumed that the chronic inflammatory response to Chlamydia is modulated by the immune system. It has been experimentally demonstrated that the HSP60 is an important factor in the delayed type of hypersensitivity (12). It has also been shown that the serological response to HSP60 is connected with chronic infection of the pelvic organs (PID) in serologically-positive patients for Chlamydia (3, 6, 11). This supports the idea that the response to chlamydial HSP60 is crucial in the immunopathogenesis of female genital inflammations (13, 14).

The relation of anti-cHSP60 antibodies to the failure of in-vitro fertilization (IVF) becomes even more evident in cases of women suffering from pathological changes caused by chlamydial infections. Anti-HSP antibodies (HSP70 in this case) were found in 43.1% of women with tubal occlusion and in 41% of women with hydrosalpinx-(13). Other authors have examined 122 women from an IVF program for the IgA antibodies in cervical secretions produced against synthetic peptides corresponding to the epitopes of the chlamydial HSP60 antibody (14). Their amino acid sequence in position 260-271 was determined to be the immunodominant and it is significant that this epitope is present both in human and chlamydial HSP60. The authors of this study (14) detected the chlamydial antibodies in follicular fluid, which were in turn in positive correlation with the antigen of human HSP in this same fluid. It turned out that all women with expressed HSP60 suffer from tubal blockage and that IVF in their cases has failed.

The results obtained seem to confirm the dominant role of *C. t.* in originating the antibodies anti- cHSP60. This finding is in concordance with those authors (6, 8, 9), who have proved that the anti *C. t.* and anti *C. p.* antibodies occur in women with tubal infertility. Also other authors of the same opinion have confirmed that a cross reaction between *C. t.* and *C. p.* does not have an influence on the prevalence of the anti- cHSP60 antibodies (10).

Our findings correspond to those of other authors (14, 15, 16). It is evident that infection with *Chlamydia trachomatis* and the subsequent high sensitivity induced by the chlamydial shock protein indicate an unfavorable prognosis for reproduction potential and minimizes the chances of successful IVF.

Conclusion

C. trachomatis has a significant impact on the production of antibodies against cHSP60. This fact can be documented by the considerably higher occurrence of the specific antibodies against *C. trachomatis* in women with a positive finding of antibodies against the cHSP60, in contrast to those women without such antibodies. Preceding infection with *C. trachomatis* and following

sensitization with chlamydial heat shock protein indicate an unfavorable prognosis for reproduction and impairs the prospect of a successful in-vitro fertilization. It is suggested that antibodies against chlamydial HSP60 can be recommended as a further auxiliary criterion in women suffering from fertility disorders.

REFERENCES

1. Lindquist- S. The heat-shock response. Ann Rev Biochem 1986; 55: 1151-91.

2. Rittosa F. A new puffing pattern induced by temperature shock and DNP in Drosophila. Experimentia (Basel) 1962; 18: 571-3.

3. Dieterle S and Wollenhaupt J. Humoral immune response to the chlamydial heat shock proteins hsp60 and hsp70 in Chlamydia-associated chronic salpingitis with tubal occlusion. Hum Reprod 1996; 11: 1352-6.

4. Simms I. Epidemiology. In: Moss, T.R. ed. International Handbook of Chlamydia. Exeter, UK, 2001; 1-11.

5. Moseley PL. Heat shock proteins and the inflammatory response. Ann N Y Acad Sci 1998; 856: 206-13.

6. Land JA and Evers JLH. Chlamydia infection and subfertility. Best Pract Res Clin Obstet Gynaecol 2002; 16: 901-12.

7. Pospisil L, Canderle- J, Medkova Z et al. Antibodies against the chlamydial heat shock protein (cHSP60) in blood sera and seminal plasma of men (male partners of subfertile couples, and donors of semen, and blood donors. Derma (Bratislava - Prague) 2003; 3 (4): 4-8 (Czech).

8. Freidank HM, Clad A, Herr AS. et al. Immune response to *Chlamydia trachomatis* heat shock protein in infertile female patients and influence of *Chlamydia pneumoniae* antibodies. Eur J Clin Microbiol Infect Dis1995; 14: 1063-9.

9. Saikku P. Chronic *Chlamydia pneumoniae* infections. In: Chlamydia Research. Stary A. ed. Proceedings of the 3rd Meeting of the European Society for Chlamydia Research, Vienna, Austria, 11.-14. Sept., 1996; 215-8.

10. Persson K, Osserr S, Birkelund S et al. Antibodies to *Chlamydia trachomatis* heat shock proteins in women with tubal factors infertility are associated with prior infection by *C. trachomatis* but not by *C. pneumoniae*. Hum Reprod 1999; 14: 1969-73.

11. Neuer A, Spandorfer SD, Giraldo P et al. The role of heat shock proteins in reproduction. Hum. Reprod. Update 2000; 6: 149-59.

12. Morrison RP, Belland RJ, Lyng K et al.: Chlamydia disease pathogenesis: the 57-KD chlamydial hypersensitivity antigen is a stress response protein. J Exp Med 1989; 170: 1271-83.

13. Spandorfer SD, Neuer A, LaVerda D et al. Previously undetected Chlamydia trachomatis infection, immunity to heat shock proteins and tubal occlusion in women undergoing in vitro model that simulates in vivo events. Fertil Steril 1999; 71: 619-26.

14. Witkin SS, Jeremias J, Neuer A et al. Immune recognition of the 60 kDa heat shock protein: implication for subsequent fertility. Infect Dis Obstet Gynecol 1996; 4: 152-9.

15. Neuer A, Lam N, Tiller F-W et al. Humoral immune response to membrane components of Chlamydia trachomatis and expression of human 60 kDa heat shock protein in follicular fluid of IVF patients. Hum Reprod 1997; 12: 925-9.

16. Witkin SS, Spandorfer S, Giraldo P et al. Cervical antibodies to Chlamydia trachomatis, the Chlamydial 10 kDa heat shock protein (cHSP10) and the human 60 kDa heat shock protein (hHSP60) and adverse in vitro fertilization (IVF) outcomes. Proceedings of the 10th Int. Symp. On Chlamydial Infection, Antalya, Turkey, 16.-21. June, 2002; p. 324.

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