

ORIGINAL ARTICLE

A PROSPECTIVE STUDY TO ANALYZE THE SPECIFICITY OF CHLAMYDIAL HEAT SHOCK PROTEIN (CHSP60) ANTIBODIES TO DIAGNOSE TUBAL INFERTILITY

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ABSTRACT

The aim: To investigate the utility of testing for chlamydial heat shock protein 60 (CHSP60) antibodies in the diagnosis of tubal infertility.**Materials and methods:** All the collected samples were assayed for IgM and IgG antibodies to chlamydia trachomatis and *chlamydial heat shock protein 60* (CHSP60) by using immunofluorescence and enzyme-linked immunosorbent assay (ELISA) techniques, respectively.**Results:** There were no substantial differences between antibodies to *C. trachomatis* in females with tubal infertility (67%) and non-tubal infertility (48%). However, women with tubal infertility (45%) have more anti-CHSP60 antibodies than non-tubal infertility (9%). Antibody screening for *C. trachomatis* has only (63%) sensitivity and (54%) specificity for detecting tubal infertility. On the other hand, the CHSP60 antibody testing has (44%) sensitivity and 92% specificity for diagnosing tubal infertility. A positive microimmunofluorescence (MIF) titer was observed in 12 of 18 (67%) females with the tubal problem, 31 of 64 (48%) with non-tubal infertility ($P=0.3$, $OR=2.2$, 95% $CI=0.71$ to 8.01). The CHSP60 antibodies were found in 8 of 18 (45%) females with tubal problem & 6 of 64 (9%) women with non-tubal infertility, power factor alpha $P=0.004$, $OR=9.3$, 95% $CI=2.1$ to 43.2, power= 1.002 for $n=0.05$). Incorporating CHSP60 and *C. trachomatis* antibodies testing gives an excellent positive probability proportion of 10 to diagnose *C. trachomatis* associated tubal infertility.**Conclusions:** CHSP60 antibody testing is a more specific evaluation than antibody testing for *C. trachomatis* for predicting *chlamydia*-associated tubal infertility. Using these tests at the first infertility examination may help the immediate diagnosis for non-interceptive tubal infertility.**KEY WORDS:** tubal infertility, tubal occlusion, chlamydial heat shock protein (CHSP60), *Chlamydia trachomatis*, antibodies

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INTRODUCTION

Infection with *Chlamydia trachomatis* is an important sexually transmitted condition related to tubal infertility with increased salpingitis episodes that leads to tubal blockage [1-3]. Many studies revealed that serologic evidence of previous *Chlamydia* infection is exceptionally related to tubal infertility [4-8] and also decreased the success rate of a positive outcome for in vitro fertilization (IVF) [9]. The individuals with positive serology to *chlamydia* are at higher risk of developing the pelvic inflammatory disease (PID) [10, 11].

It was suggested that chlamydial antigen might trigger the pro-inflammatory response in host immune cells [12]. The oxidative damage to the DNA and decreased antioxidants concentration may be associated with *chlamydia*-induced tubal damage [13]. The immunopathology seen in genital tract infections is similar to those elicited by the chlamydial conjunctival infection resulting in scarring trachoma. The reinfection is determined as an essential risk factor in the pathogenesis of trachoma development [14].

The mechanism by which chlamydial infection results in

tubal damage have been studied however stays uncertain. Primate research studies recommend that reinfection with *C. trachomatis* may be the essential part of chronic salpingitis, causing distal tubal blockage [15].

The serological analysis and diagnosis for *chlamydial* antibodies early point can be a prognostic tool to estimate the risk of CT-related complications and prevent late complication development. Recent research studies have shown a strong association between antibody action to the chlamydia heat shock protein 60 (CHSP60) and ectopic pregnancy [16]. Furthermore, seropositivity to human HSP60 decreases the chance for ectopic gestation in individuals with previous chlamydial infection [17], along with the advancement of chlamydia-associated tubal infertility [18].

Chlamydial heat shock protein is a homolog of the gro-EL family of heat shock proteins [19, 20]. This family of proteins is highly conserved among both eukaryotes and prokaryotes [21, 22]. It has been recommended that antibodies versus conserved epitopes on CHSP60 might cross respond with those of hHSP60 and initiate an autoimmune inflammatory response [23-27].

Table 1. Summary of individuals included in this study

SR No	Pathology	Sample size (n=82)
1	Tubal infertility	Age= 34.2 ± 2.4 years, (n=18)
2	Non tubal infertility	Age= 32 ± 5 years, (n=64)
	Associated with:	
I	Male oriented	14
II	Ovulatory Dysfunction	16
III	Combined Male And Ovulatory Dysfunction	18
IV	Idiopathic	17

A research study identified serum antibodies testing to the entire *C.trachomatis* organism as more precise than hysterosalpingography (HSG) to predict tube-related infertility [5]. We compared *chlamydia* serology and antibodies accuracy versus CHSP60 in predicting infertility diagnosis related to tubal factors in a prospective study.

THE AIM

This study is aimed to investigate the effectiveness of chlamydial heat shock protein 60 (CHSP60) antibodies test and to analyze the specificity and sensitivity of CHSP60 measured by ELISA in comparison with standard chlamydial antibodies measurement with other diagnostic techniques like microimmunofluorescence (MIF) in the diagnosis of tubal infertility in a prospective study.

MATERIALS AND METHODS

After the suggested research study with permission approved by the Study Ethics Board at the Obstetrics and gynecology post-graduation department, Bogomolets national medical university, Kyiv, Ukraine, sample were obtained from 82 unselected females presenting for first infertility examination in the infertility center at the gynecology department, Bogomolets national medical university, Kyiv, Ukraine. All women had necessary examinations performed, consisting of basal body temperature charting, and/or mid-luteal, and/or late luteal endometrial biopsy, cervical *C. trachomatis* screening with ELISA or polymerase chain reaction assays. The medical diagnosis of tubal infertility made by HSG and/or laparoscopic test exposing distal tubal blockage or laparoscopic evidence of peritubular adhesions. A laparoscopic assessment was not performed if the complete reciprocatory distal tubal obstruction was diagnosed on HSG. All collected samples were assayed for immunoglobulin IgG and IgM antibody to *C. trachomatis* by the microimmunofluorescence (MIF) approach of Wang, also Grayston [28] utilizing detoxed Formalin-fixed primary bodies.

Sera were assessed at a dilution of 1:8 and were tittered at double dilutions to the endpoint. *Chlamydia trachomatis* seropositivity was defined as a MIF titer of n 1:8 [29]. An ELISA utilizing recombinant CHSP60 expressed as a mixed protein with glutathione-S-transferase, as an anti-

gen, was used to analyze antibodies existence to CHSP60 as described previously [18, 30] patient sera were diluted 1:500 in addition to incubated with recombinant antigen bound to 96-well microtiter plates. Horseradish peroxidase-conjugated goat anti-human IgG was added, and the optical density of each was well determined. All sera favorable by ELISA were confirmed by immunoblotting using recombinant CHSP60 as antigen. Sera were checked blindly and without the information of clinical diagnose.

Groups were contrasted by χ^2 or Fisher's exact test. Odds ratios (OR) with 95% confidence intervals (CI) calculated probability ratios are also computed to help the diagnostic precision of both serologic tests for their ability to anticipate tubal disease as the reason for infertility the patients came for infertility assessment. The computation of possibility ratios makes it possible to compare the diagnostic importance of tests independent of infection frequency in varying populations. The ratio for positive test calculated as sensitivity/(100 — specificity). A positive possibility of 2 to 5 recommends a non-satisfactory clinical test, 5 to 10 shows a good scientific test, and > 10 is an excellent clinical test. The ratio for the negative test was also calculated as sensitivity/(100 — specificity). The unfavorable ratio of 0.5 to 0.2 shows an unsatisfactory examination, 0.2 to 0.1 is a good clinical assessment, and value <0.1 shows a superb clinical assessment [31].

RESULTS

Eighty-two (82) women were included in our research, 18 with the final diagnosis of tubal disease, 2 of those with tubal-associated problems and additionally had ovulatory dysfunction, 3 with male-oriented issues, and 3 with both male-oriented issues along with ovulatory dysfunction in the development of tubal infertility. Sixty-four women with non-tubal infertility: 14 with male variable, 16 with an ovulatory disorder, 18 with combined male factor in addition to ovulatory dysfunction, and 17 with idiopathic infertility the mean age of the women with tubal infertility was (34.2 ± 2.4 years) in comparison to those with different other causes of infertility (32 ± 5 years) as shown in Table 1.

An overall of 8 women had a history of pelvic inflammatory disease (PID), only three with tubal conditions. Consequently, 14 of 17 women with the tubal problem in our collection had no previous background of PID.

Table 2. Simple *C. trachomatis* antibodies analysis by MIF screening assay to predict tubal infertility

Tubal VS non-tubal infertility	Sample % age with positive MIF titer to <i>C. trachomatis</i> antibodies	Calculated values	MIF Sensitivity for <i>C. trachomatis</i> antibodies	MIF Specificity for <i>C. trachomatis</i> antibodies	Prediction value		Probability ratio	
					positive	negative	Favorable	unfavorable
	1. Tubal 12 of 18 (67%)	P=0.3 OR=2.2	65%	58%	29%	88%	1.15	0.71
	2. non-tubal 31 of 64 (48%)	95% CI=0.71 to 8.01						

P= power factor alpha, OR=odds ratio, CI=confidence interval, *C. trachomatis*=*Chlamydial trachomatis*

Table 3. CHSP60 antibodies measurement (nonspecifically) by ELISA in all the individuals with the tubal and non-tubal cause of infertility

Tubal VS non tubal infertility	Sample % age positive to CHSP60 antibodies by ELISA	Calculated values	CHSP60 antibodies testing Sensitivity	CHSP60 antibodies testing specificity	Probability ratio
	1. Tubal infertility 8 individuals from 18 (45%)	P=0.004 OR=9.3	44%	93%	6.1
	2. nontubal infertility 6 individuals from 64(9%)	95% CI=2.1 to 43.2 Power =1.002 for n=0.05			

P= power factor alpha, OR=odds ratio, CI=confidence interval, CHSP60=chlamydial heat shock proteins, ELISA=enzyme linked immunosorbent assay

Table 4. CHSP60 Antibodies measurement specifically in individuals with tubal infertility and with positive MIF titer in comparison with non-tubal infertility or negative MIF titer

Tubal VS non tubal infertility	Sample % age positive to CHSP60 antibodies by ELISA	Calculated values	CHSP60 antibodies testing Sensitivity for <i>C. trachomatis</i>	CHSP60 antibodies testing specificity for <i>C. trachomatis</i>	Prediction value		Probability ratio
					positive	negative	
	1. tubal 8 of 12 (67%)	P=0.0000412 OR=31.02	70%	93%	58%	86%	10
	2. non-tubal 7 of 70 (10%)	95% CI=7 to 220					

P= power factor alpha, OR=odds ratio, CI=confidence interval, *C. trachomatis*=*Chlamydial trachomatis*, CHSP60=chlamydial heat shock proteins

Thirteen individuals were located to have endometriosis at the time of laparoscopy, and 10 of these women without any ovaries or tubes related problem. Eight women had a prior background of ectopic pregnancy, and likewise, three of these females have normal tubes at laparoscopy after the resolution of the ectopic gestation (treated with methotrexate or direct salpingostomy). The remainder of our selected individuals diagnosed infertility with male issues or related to ovulatory aspects. A positive MIF titer in 12 of 18 (67%) women with the tubal issue and 31 of 64 (48%) with various other causes of infertility (P=0.3, OR = 2.2, 95% CI = 0.71 to 8.01). Hence, screening assessment for tubal conditions, MIF has a sensitivity of 65% and specificity of 58%, with a positive prediction of 29% and the negative prediction of 88%. Calculation of probability ratio revealed that MIF screening for *C. trachomatis* antibodies is not a helpful medical test for the prediction of the tubal problem in the individual with infertility (favorable possibility proportion = 1.51 as well as unfavorable chance ratio = 0.7), as shown in table 2.

The CHSP60 antibodies found in 8 of 18 (45%) women with the tubal problem in addition to 6 of 64 (9%) women with other causes of infertility, power factor alpha α P=0.004, OR = 9.3, 95% CI = 2.1 to 43.2, probability ratio=6.1, power = 1.002 for n = 0.05). Consequently, as an indicator of tubal infertility, CHSP60 antibody testing has a sensitivity level of 44% and specificity of 93%, as shown in table 3.

For diagnosis of *Chlamydial* linked tubal problems (specified as the tubal condition with a positive MIF titer), the CHSP60 test performed in 8 of 12 (67%) patients with tubal disease in addition to 7 of 70(10%) females with other causes of infertility or with a negative MIF titer (P=0.0000412, OR =31.02%, CI = 7 to 220). Therefore, the level of sensitivity of the CHSP60 assay for discovering *C. trachomatis* linked tubal disease is 70%, along with its 93% specificity. The positive value of prediction for the CHSP60 assessment for the medical diagnosis of tubal infertility is 58% as well as the negative value of prediction is 86%, as shown in Table 4. A person with the tubal condition is 6.1 times more likely than women without

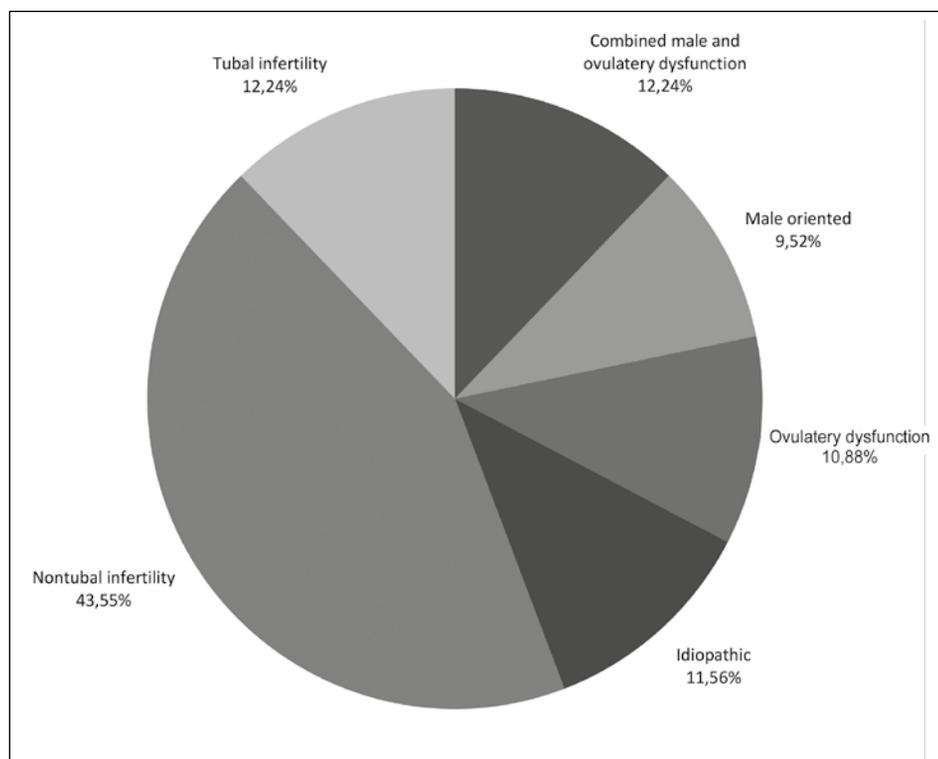


Fig. 1. Causes of infertility in a sample of the population.

tubal problems to have antibodies to CHSP60 (positive probability proportion).

The structure of tubal and non-tubal causes of infertility in the population sample selected for this study is presented in Figure 1.

DISCUSSION

CLINICAL FINDINGS

The determination of CHSP60 antibodies was a more accurate and particular technique to identify tubal infertility associated with *C. trachomatis* infection. The MIF was a more sensitive but less specific diagnostic method.

It has been recommended that *C. trachomatis* antibody screening might be valuable as a testing parameter in diagnosis for tubes related infertility [32]. As a forecaster of tubal condition, we located that the MIF for *C. trachomatis* test has limited specificity of merely 58% and a level of sensitivity of 65% for anticipating tubal condition in our center population.

The negative and positive ratios of 1.51 and 0.71, respectively, indicate the low sensitivity of MIF for diagnosis of tubes associated problems. The seroprevalence of *C. trachomatis* in most grown-up populations is 40% to 60% [33]. The MIF assessment permits a useful and more specific discovery of antibodies against different chlamydial strains [34]. Furthermore, Dabekausen et al.'s [5] interpretation of tubal disease consisted of tubo-peritoneal adhesion in addition to independent tubal pathology seen at laparoscopy. In contrast, in our collection, we specified tubal condition as the direct exposure of bilateral distal tubal clog or considerable peri tubal adhesion.

The degree of sensitivity of this evaluation for finding tubal condition is negative; the 92% specificity of this test for finding tubal condition makes it an important assay. The favorable probability proportion of CHSP60 antibodies screening found out the presence of the tubal condition in an unselected population of individuals providing for infertility was 6.1, making it an outstanding examination for predicting tubal presence associated infertility. The CHSP60 antibodies screening will help reveal the tubal damages resulting from *C. trachomatis* infections and help differentiate with other infertility causes.

Brunham et al. [23] previously reported that 19 of 21 (91%) females with ectopic pregnancy along with seropositive for *C. trachomatis* had antibodies to CHSP60. Three of these four women had CHSP60 antibodies (75%) Just 4 of these seven females had an apparent tubal problem.

STRENGTHS AND LIMITATIONS

This study's strengths are selecting individuals, especially those with the infertility issue associated with tubal factors and full patient background (e.g., age, BMI, ethnic background, essential language, gravidity and parity, other comorbidities, previous infections, present medications). This research study is a suitable reevaluation for tubal infertility risks evaluation in the Kyiv, Ukraine population and also contributes to literary works on the prospective impact on the female with infertility connected with fallopian tubes.

Limitations of the study included the prospective case-control nature of the study, restricting the data collection. The sample size was also very limited as only those individuals were included whose infertility problem was explicitly associated with fallopian tubes.

The data was collected only from three institutes. There are regularly institutional propensities in providing medical diagnosis and treatment to women, and duplicating this research study with multiple research studies would undoubtedly reinforce our results' generalizability.

Women with tubal infertility, age less than 40 years or more than 25 years, with ovulatory dysfunction, endometriosis, ectopic pregnancy, and women with routine gynecological check-up were included.

Females with a previous history of autoimmunity, oncology, or hypersensitivity were excluded.

RESEARCH IMPLICATION

It is not well understood that CHSP60 antibodies in serum itself a reason for tubal infertility, or it sets off the activation of a few other chemical cascades in the body that results in this pathology's growth. Further immunochemical studies need to be done on this parameter. Given the exploratory nature of the results, we await verification of our research from future research studies. We also hope to discover associations amongst tubal infertility and various other kinds of heat shock proteins (HSP) i. e HSP 10, 70, 90, 110, and the decision of specific criterion to diagnose the linked etiology.

CLINICAL IMPLICATION

Among the essential factors for screening for *chlamydia*, unlike other sexually transmitted disorders as a cause of the tubal problem, interestingly infertility is the significant outcome of genital *chlamydia* infections in women, and mostly these infections are asymptomatic. Non-treated and ignored infections can spread into the upper genital system and likewise trigger PID (pelvic inflammatory disease) with resultant ectopic gestation as well as tubal occlusion and other complications. Numerous study studies have shown that most women with the tubal cause of infertility did not mind a history of *chlamydia* infection [4]. These examinations would undoubtedly be a fast and definite helpful approach for establishing the diagnose in these women without various other unwanted and expensive interventive treatment and diagnostic measures.

OUTCOME

The performance of the CHSP60 antibodies assessment depends upon its high specificity (92%) and good positive ratio (6.1) for the presence of tubal infertility. The negative MIF analysis has an excellent (85%) negative value of prediction that makes it useful alone in diagnosing tube-related infertility.

A positive MIF assay combined with CHSP60 antibody screening generates an extraordinary *C. trachomatis*-linked tubal infertility (good possibility ratio= 10). The CHSP60 antibodies evaluation and MIF testing should exist as an analysis tool as part of the first infertility examination.

The women with a positive CHSP60 antibody assay might consider moving into IVF-ET treatment instead of finding extra interceptive and expensive screening examination, mainly IVF-ET seems specifically reliable in treating tubal-associated infertile females that have antibodies to the CHSP60 [34].

CONCLUSIONS

The *Chlamydia Trachomatis* infection is one of the significant risk elements for the development of tubal infertility. The measurement of CHSP60 antibodies by ELISA in serum samples is a particular extra parameter to detect tubal infertility related to chlamydial trachomatis infection instead of primary antibody *C. Trachomatus* as well as using a less specific microimmunofluorescence (MIF) strategy. The use of both methods together at the preliminary diagnostic evaluation of women may help provide the prompt diagnosis as MIF has a lot more sensitivity and CHSP60 antibodies by ELISA is extra particularly specific for medical diagnosis of *Chlamydia Trachomatis* linked tubal infertility.

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Conflict of interest:

The Authors declare no conflict of interest.

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