Original Article

# Detection of Chlamydial Heat Shock Protein 60 and 10 Antibody among Female Infertility

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## Abstract

**Introduction:** Of the many sexually transmitted pathogens, *Chlamydia trachomatis* is increasingly being associated with long-term sequelae such as infertility, apart from causing genital tract infections. Many inflammatory responses directed against chlamydial infection can cause tubal damage resulting in infertility. For example, chlamydial heat shock protein 60 (cHSP60) and cHSP10 along with humoral immune response. The aim of our study is to detect the presence of immunoglobulin G (IgG) antibodies against Major Outer Membrane Protein (MOMP), cHSP60, and cHSP10 among female infertility. **Methods:** A total number of 230 female infertility patients attending the Outpatient Department of Reproductive Medicine, SRIHER, were included in the study. Detailed history documented in the proforma. Serological detection of *C. trachomatis* IgG antibody against MOMP, cHSP60, and cHSP10 antibody was done by enzyme-linked immunosorbent assay (ELISA). **Results:** *C. trachomatis* IgG antibody against MOMP was detected in 15 (6.5%) of 230 females. High seropositivity to cHSP60 antibodies was detected among females of tubal factor infertility (TFI). Our study showed that cHSP60 antibodies (3.4%) were more common than cHSP10 (2.6%). **Conclusion:** Our study suggest cHSP60 or cHSP10 antibody detection by ELISA along with TFI is helpful for diagnosis and early institution of therapy. The accuracy of TFI prediction could be increased by the detection of anti-MOMP and cHSP60 over cHSP10 among secondary infertility than primary. The most probable reason for high seropositivity among secondary infertility patients may be due to repeated infection and chronicity because of longer active sexual life.

**Keywords:** *Chlamydia trachomatis,* enzyme-linked immunosorbent assay, heat shock protein, tubal factor infertility

## Introduction

Globally, *Chlamydia trachomatis* infection (CTI) is the most common bacterial sexually transmitted disease, with over 130 million new instances being reported every year.[1] Of the sexually active age groups of the general population, around 10 million cases of asymptomatic genital chlamydial infection have been recorded in India.[2] The literature demonstrated that about 23.3%–33% of cases of *C. trachomatis* were found in India.[2,3] Over 80% of these individuals remain asymptomatic and may therefore remain undiagnosed. A persistent infection with *C. trachomatis* can spread to the upper genital tract and resulting pelvic inflammatory disease (PID). If left untreated this might result in long-term sequelae like infertility.[4] Infertility is defined as failure to conceive following a year of regular unprotected sexual intercourse. It can be categorized as primary and secondary. Couples who have never conceived are considered to be primary infertility, whereas secondary infertility is defined as a couple who are unable to conceive following a previous successful conception but not necessarily having a live birth.[5] Other than chlamydial infection, infertility can also be caused by several factors such as tubal damage (blocked tubes), ovarian disorder (polycystic ovary syndrome and other follicular disorders), uterine disorder (fibroids, septate uterus, and endometriosis), and endocrine system disorders (reproductive hormone imbalances).[6] Tubal damage or tubal factor infertility (TFI) is multifactorial, and it can be caused by bacterial infection (*Neisseria gonorrhoeae, Mycoplasma genitalium,*

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and *Mycobacterium tuberculosis*), fallopian tube injury due to past surgery, polyps, endometriosis, ischemic nodules, tubal spasm, congenital abnormalities, and peritoneal factors such as peritubular adhesions, altered tubal motility, and fimbrial end blockage.[7] According to the WHO, 18%–20% of infertile women globally have CTI.[8] Based on several reports, 28%–30% of infertile women in India have CTI, which is a significant percentage considering the global situation.[8] There is a strong epidemiological, histological, and serological association between chlamydial upper genital tract infection; PID has been found in several case–control studies and cohort analyses.[9-12] According to several seroepidemiological investigations, the development of PID, ectopic pregnancy, and TFI has been consistently linked to antibody responses to the major outer membrane protein (MOMP), chlamydial heat shock protein 60 (cHSP60), and cHSP10.[2,4,10] Many women with TFI can be found to have CTI and more than 70% of those with tubal occlusion have anti-*C. trachomatis* antibody.[10,13] Roughly, about 25%–35% of the people undergoing assessment for infertility have tubal illness.[12] Laparoscopy has been considered the gold standard for TFI diagnosis. However, it is expensive and invasive and may result in complications. For the diagnosis of TFI, hysterosalpingography (HSG) and hysterosalpingo sonography (HSSG) are less risky methods; however, false-positive findings are frequently observed. HSG and HSSG are also very observer-dependent and peritubal adhesions cannot be seen with these techniques. In addition, these techniques do not show impaired tubal function.[10] Increased antibody and cell-mediated immune responses to cHSP60 are seen in *C. trachomatis*-induced illness.[12,14] The usual transition of reticulate bodies to infectious elementary bodies is thought to be disrupted by this stress response, which results in long-term infection that might act as an antigenic reservoir for potentially immunopathogenic anti-heat shock protein (HSP) immune responses.[11] Repeated *C. trachomatis* inoculations in an experimental monkey model caused significant tubal damage and occlusion,[2] which raises the possibility that the host's immune response plays a role in tubal pathogenesis. It was shown in subsequent research on monkeys and guinea pigs that the exposure to cHSP60 caused a delayed hypersensitive reaction and pronounced localized inflammation.[2,15] Immunodominant MOMP of *C. trachomatis* is a target of neutralizing antibodies and serotyping antigens. Specific MOMP variants are associated with upper genital tract infection and antibodies to the antigen were reported to be higher in patients with occluded tubes.[9] During chronic infection, *C. trachomatis* expresses TroA and HtrA proteins. Immunoglobulin G (IgG) antibody responses to these proteins are more common in patients with recurrent chlamydial infections than healthy controls. Compared to subfertile women with tubal block, women with TFI are more likely to have IgG antibodies against TroA and HtrA.[10] Further, the pathogenic consequences of persistent chlamydial infection and tubal blockage are linked to immunological response to cHSP10.[16] Coexpression of cHSP60 and cHSP10 was also documented.[2,9,11] According to the investigations by Akande *et al*. and den Hartog *et al*., positive *C. trachomatis* serology has been linked to tubal damage.[17,18] To identify subfertile women at the greatest risk of tubal disease, *C. trachomatis* IgG antibody testing has been employed in the infertility workup. Hence, the present study is undertaken to detect the presence of IgG antibodies against MOMP, cHSP60, and cHSP10 among females with infertility by enzyme-linked immunosorbent assay (ELISA).

## Methods

### Study population

A cross-sectional study with 230 women attending the Outpatient Department of Reproductive Medicine and Surgery, Sri Ramachandra Hospital, SRIHER, a tertiary care hospital in Chennai were included according to the inclusion and exclusion criteria.

### Inclusion criteria

- Infertile women in the reproductive age group between 18 and 42 years of age with both primary and secondary causes
- No history of genital tuberculosis
- Patients who consented to the study.

### Exclusion criteria

- History of antibiotics treatment in the previous 2 months
- Recently treated genital tuberculosis
- Not willing to consent.

The sample size was calculated by the odds ratio of 2.5% with a power of 90% and $\alpha$ error of 5%. This study was cleared by the institutional ethics committee with the enrollment number IEC-NI/20/FEB/74/11. The samples were subjected to detect antibody against MOMP, cHSP60, and cHSP10 by ELISA, and significance was derived by statistical analysis. Informed consent from all the participants was obtained after explaining the purpose of the study. A detailed clinical history was taken and documented.

### Specimen

Blood sample ($n$ = 230) was collected under strict aseptic precautions, serum was separated to detect MOMP, and cHSP60- and cHSP10-specific IgG antibodies by ELISA kits (Euroimmun, Germany and QAYEE-BIO, China) were used as per the manufacturer's instruction. The optical density was read at 450 nm using microtiter plate reader.

### Statistical analysis

Statistical analysis was done using open EPI software ("OpenEpi development is supported in part by a grant from the Bill and Melinda Gates Foundation to Emory University, Rollins School of Public Health) and Chi-squared test. A probability value of $\leq 0.05$ was considered significant and >0.05 was considered nonsignificant.

## Results

### Study participants

Two hundred and thirty infertile women with age ranging

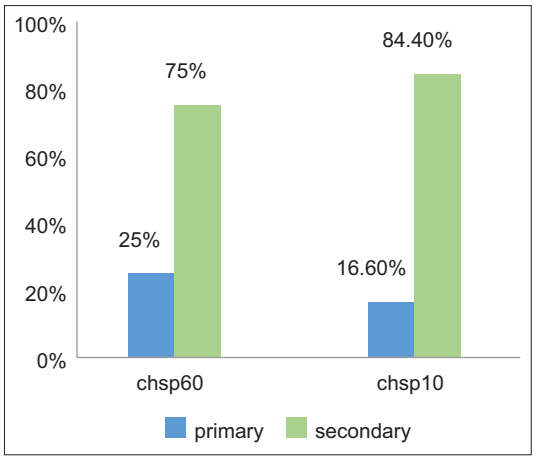
between 18 and 42 years were enrolled and tested for IgG antibodies against cHSP60 and cHSP10 by ELISA. The median age of the study participants was 28 years with an interquartile range of 6. The respective median values among primary and secondary infertility were found to be 27 and 28 years. Among the infertile cases, 7 (3.04%) were in <20 years, followed by 61 (26.5%) belonging to the age group of 21–25 years, 99 (43.0%) in the 26–30 years, 42 (18.2%) women in the 31–35 years, 15 (6.5%) in the 36–40 years of age group, and 6 (2.6%) were more than 40 years. The distribution of infertile women as per socioeconomic status is shown in Table 1.

### Seropositivity of *Chlamydia trachomatis*

ELISA results for anti-MOMP, cHSP60, and cHSP10 showed higher values in tubal block patients of *C. trachomatis* positive than *C. trachomatis* negative. Out of 230 patients, 15 (6.5%) showed ELISA positivity for anti-MOMP, 8 (3.4%) for anti-cHSP60, and 6 (2.6%) for anti-cHSP10. cHSP60 antibodies were higher in patients with tubal block than anti-cHSP10 [Table 2]. Among the cHSP60-positive patients, 2 (25%) and 6 (75%) were primary and secondary infertility, respectively. Similarly, 1 (16.6%) primary infertility and 5 (83.3%) secondary infertility were from cHSP10-positive patients [Graph 1]. The distribution of the enrolled women as per IgG seropositivity is shown in Table 3.

### Correlation between anti-major outer membrane protein with anti-chlamydial heat shock protein 60 and anti-chlamydial heat shock protein 10

A significant positive correlation was seen between anti-MOMP ($P$ = 0.0264), anti-cHSP60 (0.0369), and cHSP10 (0.0263) in female infertility patients. In the *C. trachomatis*-infected (confirmed by MOMP) cHSP60 ELISA-positive ($n$ = 8, 3.4%) patients, 5 (62.5%) had PID, 7 (87.5%) had tubal block, and 4 (50%) had cervicitis. In the cHSP10 ELISA-positive ($n$ = 6, 2.6%) patients, 3 (50%) had PID, 1 (16.6%) had tubal block, and 4 (66.6%) had cervicitis. cHSP60 antibody results in significantly increase in tubal block patients when compared to other *C. trachomatis*-positive patients. Correlation between MOMP and cHSP60 antibody was statistically significant in tubal block patients than cHSP10 [Table 4].




**Graph 1:** Distribution of type of infertility

## Discussion

Infertility is increasingly being recognized as a global health problem, including India.[19] The upper genital tract infection caused by *C. trachomatis* frequently has no symptoms.[10] Cervical CTI that is undiagnosed or untreated takes a chronic course. The bacteria pass through the endometrial cavity to reach the upper genital tract, where it can cause various immunopathological changes which ultimately result in infertility.[4,10] Studies have shown increased *C. trachomatis* MOMP IgG and immunoglobulin A levels have been linked to upper genital tract infections and patients with occluded tubes.[2,20,21] In our study, the *C. trachomatis* MOMP IgG antibodies have been shown to be high in patients with occluded tubes. There has been a substantial correlation between TFI and circulating chlamydial antibodies reported by Dutta *et al*.[2] In our study, the antibodies to *C. trachomatis* and TFI ($P$ = 0.0080) are statistically correlated. According to the study by Dutta *et al*., Srivastava *et al*., and LaVerda *et al*. comparing to acute lower genital tract infections, immunity to *C. trachomatis* HSP60 and HSP10 is more frequently linked with chronic upper genital tract infections and fallopian tube damage.[2,11,22] In the present study, anti-cHSP60 and 10 are frequently associated with upper genital tract infections than lower genital tract. Although the existence of cHSP antibodies has been previously documented, the association between disease and seroprevalence among patients with cervicitis and infertility is largely unexplored in India. According to several studies, seropositivity for HSP60 antibodies is associated with higher rates of salpingitis, pelvic adhesions, prior PID, and infertility,[2,4,10] and correlating to our present study, the seropositivity for cHSP antibodies is associated with high rates of PID, cervicitis, tubal block, and infertility. Therefore, it is imperative to the early screening of women with infertility. In addition, it has been noted by Beatty *et al*. in subclinical chlamydial infection; the ratio between cHSP60 and MOMP rises substantially.[23] A known characteristic of

**Table 1: Distribution of infertile women as per socioeconomic status (modified B G Prasad Scale 2022)**

| Group | Class I | Class II | Class III | Class IV | Class V | Total | $\chi^2$, DF, $P$, $r$ |
|---|---|---|---|---|---|---|---|
| Primary | 1 | 27 | 63 | 46 | 2 | 139 | 10.585, 0.0316, 0.93 |
| Secondary | 1 | 5 | 44 | 37 | 4 | 91 | |

The $P$ value was statistically significant and the majority of the participants belonged to the middle socioeconomic group. DF: Degree of freedom

*C. trachomatis* is the coexpression of cHSP60 and cHSP10, and studies have shown a positive association between the two HSPs in *C. trachomatis*-positive samples and a negative correlation in *C. trachomatis*-negative individuals by Dutta *et al*.[2,9] In our study also, a similar association was seen in *C. trachomatis*-positive patients. Dutta *et al*. stated that human HSP60 is a highly conserved protein that shares 48% amino acid sequence similarity with cHSP60, and particular antibodies have been discovered to cross-react with human HSP60; it is possible that these antibodies may contribute to disease etiology.[2,9] Host HSP60 production is induced during fast chlamydial cell growth or differentiation or following environmental stress like inflammation. Immunological tolerance may be broken down by prolonged or recurrent exposure to cHSP60, which might result in self-HSP60 directed immunity through cross-reactive T-cell and B-cell epitopes.[16] A research by Ault *et al*. and Kinnunen *et al*. noted that the activation of an immune response that targets self-HSP60 is thought to be detrimental to the well-being of the host, and it is linked to a change from a protective immune response to a pathogenic response.[14,24] According to the current study, women with secondary infertility caused by *C. trachomatis* were more likely to have anti-cHSP60 or anti-cHSP10 antibodies than women with primary infertility. These results are consistent with earlier research by Dutta *et al*., which showed that subfertile patients with occluded tubes had high levels of cHSP60 IgG.[9] In a previous investigation, on considering polymerase chain reaction and Direct Fluorscent Assay (DFA) as a standard test of comparison, cHSP60 ELISA showed higher sensitivity and specificity for secondary infertility than primary.[9] Based on our results, cHSP60 ELISA had greater sensitivity and specificity in secondary infertile women than in primary. Our findings suggest that chronic chlamydial infection plays a significant role in the development of infertility in infected women. Our study found that cHSP60 antibodies are more accurate serological markers than cHSP10 in subfertile patients. As a result, early identification of anti-cHSP60 can serve as a reliable diagnostic and prognostic marker for infertility caused by *C. trachomatis*.

**Table 2: *Chlamydia trachomatis* positivity among tubal factor infertility**

| Group | cHSP60 | cHSP10 | $\chi^2$, DF, *P* |
|---|---|---|---|
| Group 1 | 7 | 1 | 7.0243, 1, 0.0080 |
| Group 2 | 1 | 5 | |

Group 1: CT positive with TFI, Group 2: CT positive with no TFI.
TFI: Tubal factor infertility, CT: *Chlamydia trachomatis*, DF: Degree of freedom

### Limitation

A case–control study representing different populations may give more information, and the possibility of correlation between antibody titer, clinical, and structural damage could have been done.

## Conclusion

Our study suggest cHSP60 or cHSP10 antibody detection by ELISA along with TFI is helpful for diagnosis and early institution of therapy. The accuracy of TFI prediction could be increased by the detection of anti-MOMP and cHSP60 over cHSP10 among secondary infertility than primary. The most probable reason for high seropositivity among secondary infertility patients may be due to repeated infection and chronicity because of longer active sexual life.

### Research quality and ethics statement

This study was approved by the Institutional Ethics Committee (ID: IEC NI/20/FEB/74/11). The authors followed the applicable EQUATOR Network guidelines during the conduct of this research project

### Financial support and sponsorship

Nil.

### Conflicts of interest

There are no conflicts of interest.

**Table 3: Distribution of participants as per immunoglobulin G seropositivity**

| Infertility type | IgG against MOMP | | | IgG against cHSP60 | | | IgG against cHSP10 | | |
|---|---|---|---|---|---|---|---|---|---|
| | Present | Absent | $\chi^2$, DF, *P* | Present | Absent | $\chi^2$, DF, *P* | Present | Absent | $\chi^2$, DF, *P* |
| Primary | 5 | 134 | 4.9291, 1, 0.0264 | 2 | 137 | 4.3524, 1, 0.0369 | 1 | 138 | 4.9357, 1, 0.0263 |
| Secondary | 10 | 81 | | 6 | 85 | | 5 | 86 | |

IgG: Immunoglobulin G, MOMP: Major outer membrane protein, DF: Degree of freedom

**Table 4: Chlamydial immunoglobulin G abs and hysterosalpingography as markers of tubal occlusion**

| Tubal block | CT IgG | | | cHSP60 IgG | | | cHSP10 IgG | | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive | Negative | $\chi^2$, DF, *P* | Positive | Negative | $\chi^2$, DF, *P* | Positive | Negative | $\chi^2$, DF, *P* |
| HSG positive | 9 | 50 | 9.9263, 1, 0.001629 | 7 | 52 | 16.6234, 1, 0.0004 | 1 | 57 | 0.2388, 1, 0.6250 |
| HSG negative | 6 | 165 | | 1 | 170 | | 5 | 167 | |

HSG: Hysterosalpingography, IgG: Immunoglobulin G, DF: Degree of freedom, CT: *Chlamydia trachomatis*

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