**Original Article**

# The Role of *Chlamydia trachomatis* IgG Antibody Testing in Predicting Tubal Factor Infertility in Northern Iran

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Abstract

**Background:** The purpose of this study was to investigate the role of Chlamydia serology as a screening test for tubal infertility and to compare the results with hysterosalpingography (HSG) and laparoscopic findings.

**Materials and Methods:** This was a cross-sectional study undertaken on 110 infertile women treated in the IVF Ward, at Emam Khomeini Hospital, Sari, Iran who underwent laparoscopy and HSG as part of their infertility workup.

Prior to laparoscopy, 5 ml of venous blood was drawn for measurement of serum Chlamydia IgG antibody titer (CAT). Patients' tubal status and pelvic findings were compared with CAT, as measured by microimmunofluorescence.

**Results:** Tuboperitoneal abnormalities were seen in 81.4% of seropositive patients versus 13.2% of women who were seronegative. In women with tubal damage, the numbers of positive CATs ($\geq$1:32) were significantly more than in those who had a normal pelvis (66.6% vs. 6.5%, $p<0.001$). CAT levels were higher in patients who had bilateral hydrosalpinges, bilateral tubal occlusion and pelvic adhesions (severe damage), than those with tubal distortion and unilateral occlusion (mild damage) ($p<0.05$). The positive likelihood ratio for *C. trachomatis* antibody testing was 10.28 as compared with HSG, which had a positive likelihood ratio of 3.03.

**Conclusion:** The results of this study revealed that *C. trachomatis* serology is an inexpensive and non-invasive test for tubal factor infertility screening.

***Keywords:*** Infertility, Chlamydia, Antibody, Laparoscopy, Hysterosalpingography

## Introduction

*Chlamydia trachomotis (C.trachomotis)* is the most common sexually transmitted bacterial infection worldwide, especially among young adults (1). The majority of pelvic infections caused by chlamydia are asymptomatic. Untreated chlamydia infection can cause an upper genital tract infection and pelvic inflammatory disease (PID). Chlamydial PID can cause tubal occlusion and subsequent infertility (2). Tubal pathology affects approximately 15 to 30% of subfertile women (3).

*C. trachomatis* however, is a slow (eliminate) growing intracellular organism. The growth cycle of chlamydia is 48 to 72 hours; therefore , several weeks to months are required for the growth to reach sufficient numbers to cause clinical symptoms (3). *C. trachomatis* preferentially infects the columnar epithelium. Serious sequelae often occur in association with repeated or persistent infections. The precise mechanism through which repeated infection elicits an inflammatory response that leads to tubal scarring and damage in the female upper genital tract is not yet clear (4). *C. trachomatis* may cause intraluminal adhesions, fibrosis, hydrosalpinx and pelvic adhesions. Due to the serious consequences of these conditions, *C. trachomatis* infection can affect a woman's fertility (5).

Chlamydia is now associated with at least 50% of the cases of acute pelvic inflammatory disease (PID) in developed countries. Due to the asymptomatic nature of *C. trachomatis*, the diagnosis of tubal disease cannot rely solely on the presence or absence of a history of PID. Since late sequelae of PID (chronic pelvic pain and tubal damage) have major health implications; therefore, it is important to screen this group of patients for chlamydial infection (4).

Laparoscopy and HSG are accepted methods for diagnosis of tubal damage. Laparoscopy is the gold standard for pelvic adhesions and endometriosis (4). Laparoscopy allows direct visualization of the pelvis and, in addition, tubal patency

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testing offers the opportunity to detect pelvic adhesions and endometriosis. However, laparoscopy is an invasive surgical procedure which requires general anesthesia. It is an expensive investigative procedure whose availability is limited (6). HSG is a routine procedure used as an initial investigation in many fertility centers (7). HSG in comparison with laparoscopy is less costly and risky in terms of anesthetic complications as well as organ and blood vessel damage. However, it is uncomfortable, carries a risk of ionization and is poor at diagnosing peritubal adhesions. False positive results can occur due to tubal spasms, dissimilar tubal filling pressure, excessive viscosity, faulty technique or misinterpreted films (7).

Infection with *C. trachomatis* will result in the formation of antibodies detectable in serum in chronically infected patients who have a negative test for endocervical *C. trachomatis*. In these cases, a positive serologic test may be the only indication of chlamydia involvement (8).

Previous studies have confirmed a strong correlation between positive chlamydia serologic results and salpingitis, which results in infertility (9). The severity of the disease correlates with an increase in antibody titer (10). In contrast to laparoscopy or HSG, serological detection of past chlamydia infections is non-invasive, simpler and a faster test to perform. Traditionally, micro-immunofluorescence (MIF) testing has been used to serologically test for chlamydial infection. Depending on how this test is performed, it can be used to differentiate between *C. trachomatis*, *C. pneumoniae* and *C. psittaci* infections but there is also a level of cross-reaction that occurs due to shared antigens (11). Numerous studies have reported the correlation between elevated chlamydia antibodies and tubal infertility but this test has not been widely used for screening (12). The sensitivity of chlamydia serology in detecting tubo-peritoneal damage has been demonstrated by researchers (1, 4, 6, 12-18). However, according to meta-analysis, chlamydia serology is not a better screening test than HSG (7). Also, studies by Logan et al. (19), Veenemans et al. (5), Ficicioglu et al. (20), and Gurerra-infate et al. (21), indicated that the test of chlamydia antibodies alone or in combination with HSG were not cost-effective and beneficial.

The highest prevalence of chlamydia infections are found in young adults. One of the risk factors associated with chlamydial PID is sexual intercourse at an early age (1). In Sari and its suburbs, the age of marriage is traditionally low, therefore young women in this region are at an increased risk of acquiring chlamydial PID. The purpose of our study was to investigate the role of chlamydia serology as a screening test for tubal infertility in Sari (northern Iran) and to compare the results with our HSG and laparoscopic findings. If a correlation between the chlamydia antibody titer (CAT) and tubal damage was seen, we could then perform an HSG or laparoscopy as soon as possible in those patients who had a positive CAT.

## Materials and Methods

This research is a cross-sectional study performed in the Infertility Clinic at Emam Khomeini Hospital, Sari, Iran, from 2007 to 2008. The Clinic is a subspecialty service of the Mazandaran Medical Science University. A total of 150 infertile female patients who were candidates for laparoscopy consented to participate in the present study. This study was approved by the Research Center of Mazandaran Medical University.

After giving written informed consent; routine hormonal assay, spermogram and HSG were carried out on all patients. Laparoscopy was performed on patients who had an abnormal HSG (unilateral or bilateral obstruction to the dye or abnormal dye patterns in the pelvis) or on those patients who had a normal HSG but were unable to conceive in spite of six months infertility treatment.

Patients with severe male factor infertility, thyroid dysfunction, hyperprolactinemia, serum FSH $\geq$ 15 mIU/ml, contraindications for laparoscopy (obesity, umbilical hernia), or a history of previous pelvic or abdominal surgery were excluded from the study.

Prior to performing the laparoscopy, 5 ml of venous blood was drawn for laboratory measurement of the serum Chlamydia IgG antibody CAT. All samples were evaluated by the Mazandaran Laboratory Service in Sari. An indirect microimmunofluorescence test (ANI Lab System Company, Finland), was used according to the manufacturer's instructions for IgG *C. trachomatis* titer. An IgG titer 1:32 was considered a positive result. Positive samples were serially diluted and the titers quantitated.

Laparoscopy was performed in the follicular phase of the patients' menstrual cycles without regard to the CAT result. Tuboperitoneal abnormalities were recorded by one surgeon if evidence of adhesion, endometriosis, tubal distortion, obstruction of one or both tubes or hydrosalpinges were detected. Women with severe endometriosis were excluded from analysis because their abnormalities were not caused by chlamydia.

The diagnostic value of CAT was compared with the value of HSG and laparoscopy in tubal

pathology by using likelihood ratios, and positive and negative predictive values. Calculation of a likelihood ratio (LR) will yield a score that allows categorization of test results: an LR+ of 2-5 indicates a fair clinical test, 5-10 is good, and > 10 is excellent (5).

The sampling size was based on a previously reported study. Clinical and laboratory data were analysed using SPSS software. Statistical analysis included chi-square, t test, and analysis of variance (ANOVA) which were implemented to determine the ratio of discrepancies and research methodologies. p<0/05 was considered statistically significant.

## Results

A total of 150 infertile women who underwent laparoscopic investigation were identified to participate in the study. After laparoscopy, 40 women were excluded from analysis because of severe endometriosis which was due to tuboperitoneal abnormalities not caused by *C. trachomatis*.

The womens' ages ranged from 18 to 42 years (Mean 27.5 ± 5.5 years). The duration of infertility at the time of laparoscopy ranged from 1 to 13 years (Median 3.9 ± 2 years).

A positive CAT result was seen in 27 out of 110 patients (24.5%). A CAT titer of 1:32 was seen in 15 cases (13.6%), whereas 6 cases (5.5%) had a CAT titer of 1:64 and an additional 6 cases (5.5%) had an elevated CAT of 1:128. In 33 out of 110 (30%) patients who underwent laparoscopy, tuboperitoneal damage was evident. Of 27 seropositive patients, 22 (81.4%) had tuboperitoneal abnormalities; whereas 11 out of 83 (13.2%) seronegative patients had tuboperitoneal abnormalities.

In women with tubal damage, a CAT titer of ≥1:32 was seen in 22 out of 33 (66.6%) patients, which was significantly greater than women who had a normal pelvis (6.5%; p<0.001).

Demographic data in relation to the median CAT are included in Table 1. There was not a significant relation between CAT and age, and or between CAT and duration of infertility. CAT levels were significantly higher in those women who had conceived previously as compared with primary infertile women.

CAT levels were significantly higher in women with tubal damage as seen in laparoscopy than those women without tubal damage.

In the present study, there were 26 women whose main cause of infertility was tubal damage based on laparoscopy findings and 7 women who had both tubal damage and other causes (abnormal spermogram in 3 cases and ovulatory dysfunction in 4 cases) as the reasons for their infertility.

Abnormal HSGs were seen in 20 women whose laparoscopy findings were normal. Other causes of infertility were: unexplained infertility in 25 patients, male factor in 20 patients and ovulatory dysfunction in 12 patients.

In 27 patients, a discrepancy between HSG and laparoscopy findings were noted (Table 2) and in 16 patients, there was a discrepancy between CAT and laparoscopy findings (Table 3).

***Table 1: Characteristics of women who underwent laparoscopy in relation to median CAT***

| Characteristics of infertile women | | N | Median CAT | P value |
|---|---|---|---|---|
| **Age** | < 35y | 99 | $\frac{1}{16}$ | 0/89 |
| | ≤ 35y | 11 | $\frac{1}{16}$ | |
| **Duration of infertility** | < 8y | 88 | $\frac{1}{16}$ | 0/184 |
| | ≤ 8y | 22 | $\frac{1}{16}$ | |
| **Type of infertility** | primary | 81 | $\frac{1}{16}$ | 0/037 |
| | secondary | 29 | $\frac{1}{32}$ | |
| **Tuboperitoneal abnormality** | yes | 33 | $\frac{1}{32}$ | 0/0001* |
| | no | 77 | $\frac{1}{16}$ | |

**** p < 0.05 considered statistically significant***

*Table 2: HSG compared with laparoscopic findings in 110 infertile women*

| LAP HSG | Abnormal LAP | Normal LAP | Total patients |
|---|---|---|---|
| **Abnormal** | 26 | 20 | 46 |
| **Normal** | 7 | 57 | 64 |

***Abnormal HSG: One or both tubes did not allow passage of contrast medium.***
***Abnomal Lap: Evidence of adhesion, tubal distortion, obstruction of one or both tubes or hydrosalpinx were present.***

*Table 3: CAT compared with laparoscopic findings in 110 infertile women*

| LAP CAT | Abnormal LAP | Normal LAP | Total patients |
|---|---|---|---|
| **Positive** | 22 | 5 | 27 |
| **Negative** | 11 | 72 | 83 |

***Positive: Chlamydia antibody titre≥1:32***

Positive CAT had 66.7% sensitivity and 93.5% specificity at detecting tubal disease with a positive predictive value of 80.7% and a negative predictive value of 86.7%. HSG had a 78.8% sensitivity and 74% specificity for detecting tubal disease at laparoscopy. The LR+ for the CAT test was 10.26; which indicated that a patient with tubal factor infertility was 10.26 times more likely to have a positive test result (titer >1:32) than a patient without tubal factor infertility. The LR+ of HSG was 3 (Table 4).
Table 5 shows the distribution of antibody titers for all women who underwent laparoscopy.
CAT levels were higher in patients with bilateral hydrosalpinges, bilateral tubal occlusion, and pelvic adhesion (severe damage), than in those with tubal distortion and unilateral occlusion (mild damage). The trends of increasing chlamydia antibody levels in relation to severe tubal damage was significant (p<0/05).
At laparoscopy, there were 3/61% of women with negative titers (<1:32) who had severe tubal damage. In women with the highest titers (1:128), 100% had severe tubal damage. Therefore at higher titers, a greater proportion of women are likely to have severe tubal damage than at lower titers (Table 5).

*Table 4: Comparison of CAT and HSG in 110 infertile women*

| | CAT | HSG |
|---|---|---|
| **Sensitivity (%)** | 66.7 | 78.8 |
| **Specificity (%)** | 93.5 | 74 |
| **LR+** | 10.26 | 3 |
| **LR-** | 0.35 | 0.28 |
| **NPV** | 86.7 | 89.1 |
| **PPV** | 80.7 | 56.5 |

***LR+: Positive likelihood ratio, LR-: Negative likelihood ratio, NPV: Negative predictive value, PPV: Positive predictive value***

## Discussion

In this study, we evaluated the efficiency of CAT testing to screen for tubal factor infertility and found that the prevalence of a positive CAT titer is higher in women with tubal factor infertility.
Acute genital tract infections with *C. trachomatis* can be diagnosed by direct detection of the micro-organism from the infected site. After the acute episode, the organism may no longer be detectable and chlamydia antibodies in serum may be the only indication of previous chlamydia involvement.

*Table 5: Correlation between CAT and laparoscopic findings in 110 in fertile women*

| CAT | LAP | | | | | Total patients |
|---|---|---|---|---|---|---|
| | Pelvic adhesion | Tubal distortion | Normal pelvis | Tubal unilateral damage | Tubal bilateral damage | Bilateral hydrosalpinges | |
| **1/16** | 2 (2/40) | 1 (1/20) | 72 (86/7) | 7 (8/43) | 1 (1/20) | 0 | 83 |
| **1/32** | 0 | 0 | 4 (26/6) | 10 (66/6) | 0 | 1 (6/6) | 15 |
| **1/64** | 0 | 0 | 1 (16/6) | 1 (16/6) | 2 (33/3) | 2 (33/3) | 6 |
| **1/128** | 2 (33/3) | 0 | 0 | 0 | 2 (33/3) | 2 (33/3) | 6 |
| **Total** | 4 (3/63) | 1 (0/9) | 77 (70) | 18 (16/3) | 5 (4/54) | 5 (4/54) | 110 |

***CAT: Median Chlamydia antibody titers***
***Values in parethesis are perecentages.***

The aim of screening infertile women by CAT is to identify patients with previous *C. trachomatis* infections who are at increased risk for tubal pathology. However, it has become evident that not all women develop *C. trachomatis* antibodies after a chlamydia infection and not all women with antibodies have tubal pathology (22).

Although the immunopathology underlying a chlamydia infection is poorly understood, antibody tests have been developed for clinical application. A widely used test for CAT is the MIF test which has been considered the gold standard in the serological diagnosis of chlamydial infections (23).

In the present study, the LR+ of CAT was 10.28 but the LR+ of HSG was 3.03. Positive and negative predictive values for CAT were 80.7 and 86.7, respectively. These results are in agreement with studies by Keltz et al. and Dabekausen et al. (12, 13). These results are in agreement with Dabekausen et al. and Keltz et al. as well as numerous other studies (1, 4, 6, 12-16, 24).

In a study by Veenemans et al. the CAT LR + was 1.8 and HSG was 1.7 respectively, both of which indicated a poor performance. It should be noted, however, that in this study only 48 out of the 277 patients were available for the analysis. Both HSG and laparoscopy with tubal patency testing were performed only in 48 cases, which was a smaller sample size than our study (5).

According to research by Logan et al. the sensitivity and specificity of CAT by ELSA were both lower than anticipated, with a wider confidence interval. In this study, there was less patient selection and patients with tubal damage secondary to causes other than *C. trachomatis* were included (19). The discrepancies between the findings of Logan et al. and our study may be due to CAT quantification variation.

The ELISA test tends to have a lower sensitivity and NPV, and more false negatives may be seen. Additionally, tests based on highly specific peptides may be so specific that they are not able to detect all relevant antigens (25). Consequently, highly specific tests may not be able to identify all serotypes involved in chlamydia infections thus causing false negative CAT results.

Each reference standard test has its limitations. In this study, one limitation was due to the MIF test. MIF tests are labor intensive, their readings are observer dependent and interlaboratory variation is significant (26). Therefore, in our study, two experienced laboratory technicians evaluated all samples. The possible cross-reactivity in MIF tests between *C. trachomatis* and *C. pneumoniae* antibodies is another major issue. False positive CAT results increase health care costs by increasing the numbers of laparoscopies. Therefore, if the CAT is used for selecting patients for laparoscopy, the numbers of false positive CAT results should be minimized. In our study, in order to diminish cross-reaction between different chlamydia species, the immunological activity of chlamydia lipopolysaccharide (LPS) in *C. pneumoniae* and *C. trachomatis* antigens was reduced.

Time–related antibody titer decline is a possible reason for false negative results. However, this issue may be controversial. Previous studies have suggested a chronological decline in Titers (27, 28). However, a more recent study revealed no significant decline (29). Another explanation for false negative results is the immune-mediated reaction responsible for adhesion; or, for unknown reasons, tubal occlusion may not have occurred in these women (30). Therefore false negative test results may cause expectant management. However, the strength of using this study is this fact that the decision to perform a diagnostic laparoscopy was irrespective of the result of CAT.

The strength of our study is in the fact that the decision to perform a diagnostic laparoscopy was irrespective of CAT results.

Another limitation of the CAT concerns its inability to distinguish between various sources of tubal pathology, for example: micro-organisms other than *C. trachomatis*, endometriosis, previous pelvic surgeries or peritonitis, all of which are causes of tubal infertility. In our study women with endometriosis were excluded. Therefore, in patients with menstrual dysfunction and lower abdominal pain, it is better to perform laparoscopy without regard to the CAT result.

As shown in our results, the CAT is of predictive value in detecting tubal damage. The increase in antibody titer correlated with an increased incidence of severe tubal damage as was seen in laparoscopy.

## Conclusion

*C. trachomatis* serology is an inexpensive and non invasive test for screening tubal factor infertility. In patients with a positive CAT titer greater than 1:32, the risk of tubal damage is high and an invasive investigation should be done as soon as possible.

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