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GYNAECOLOGY

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## Infertility Related Chlamydial and Gonococcal Infection in Infertile Couples in Southern Thailand

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### ABSTRACT

**Objective** To study the association between prior chlamydial and gonococcal infection and infertility, and evaluate the serum levels of IgG and IgA antibodies to *Chlamydia trachomatis* and *Neisseria gonorrhoeae* (pili) in a variety of populations.

**Design** Cross-sectional study.

**Setting** Songklanagarind Hospital.

**Subjects** The control group was pregnant women and their husbands, the comparison groups were infertile women with and without tubal occlusion and their husbands, and female commercial sex workers.

**Main outcome measures** IgG and IgA antibodies to chlamydiae and gonococci pili.

**Results** IgG antibodies to chlamydiae and gonococci pili were significantly more prevalent ( $P = 0.0073$  and  $P = 0.0260$ ) in infertile women with tubal occlusion (65.0 and 56.6%) compared with the pregnant women (44.1 and 36.7%).

**Conclusion** The data suggest that women with tubal infertility frequently have serological evidence of prior infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. These results further support the aetiological role of infection with *Chlamydia trachomatis* and *Neisseria gonorrhoeae* in tubal infertility.

**Key words :** chlamydial antibody, gonococcal antibody, infertile women, tubal occlusion

*Chlamydia trachomatis*, like *Neisseria gonorrhoeae*, is capable of causing damage to fallopian tube epithelium.<sup>(1,2)</sup> It is well recognized

that *Chlamydia trachomatis* is a major aetiological factor in female infertility. Nearly all investigators have found that more than half of the women with

documented tubal occlusion report no history of previous pelvic inflammatory disease (PID) despite serological evidence of past chlamydial infection.<sup>(3,4)</sup>

The hypothesis that sexually transmitted diseases (STD) are significantly associated with tubal infertility was supported by a large number of investigators from 14 countries who have examined the relationship between serological evidence of past chlamydial infection and tubal infertility. Despite wide variations in design, these studies have uniformly found a significant association between tubal occlusion and serological evidence of prior chlamydial infection. In addition, five of the six additional studies, which included both chlamydial and gonococcal antibodies, implied independent aetiological association between these infections and tubal infertility.<sup>(5-9)</sup>

In Thailand, the proportion of infertility directly attributes to *C. trachomatis* and *N. gonorrhoeae* is not known. Within the framework of an epidemiological study on prior chlamydial and gonococcal infection in tubal infertility, we compared antibody levels against these organisms in sera from infertile couples with and without tubal occlusion, pregnant couples, and female commercial sex workers (CSWs), as determined by enzyme-linked immunosorbent assay (ELISA).

## Materials and Methods

### Study population

Over a period of 24 months, between May 1990 and April 1992, we studied prospectively 60 infertile women with tubal occlusion and their partners, and 70 infertile women with non-tubal occlusion and their partners who attended the infertility clinic. The comparison group was 68 pregnant women with uncomplicated intrauterine pregnancies and their spouses who attended the

antenatal care clinic. In addition, 118 female CSWs who attended the STD centre, region 12, Songkla were enrolled for the study.

Female infertility was defined as an inability to conceive after more than 1 year of regular intercourse with no contraceptive use. They were thoroughly investigated to demonstrate the cause of their infertility according to the WHO Standardized Investigation of the Infertile Couple.<sup>(10)</sup> All infertile women were divided into two groups: 1) those who had tubal occlusion demonstrated by laparoscopy and chromopertubation without other organic lesions; endometriosis, fibroids, ovarian tumours, etc., and 2) those who had normal fallopian tubes or other organic lesions.

All subjects underwent a structured interview in which particular attention was paid to age, duration of marriage, age of first sexual intercourse, total number of sexual partners, and any history of genital tract infection.

### Serology

A 10 ml sample of venous blood was obtained from the various groups. The serum was separated, frozen at  $-20^{\circ}\text{C}$ , and transported to the laboratory for measurement of IgG and IgA antibodies to *C. trachomatis* and *N. gonorrhoeae*. The indirect ELISA used in this study is based on a standard WHO protocol derived from the method of Robertson et al, 1987.<sup>(11)</sup> Briefly, 100  $\mu\text{L}$  of purified elementary bodies of *C. trachomatis* serovar L1 or purified *N. gonorrhoeae* P9 alpha pili diluent were coated onto ELISA trays at a protein concentration of 1.0 g/well or 0.1 g/well, respectively. The plate was incubated overnight at  $4^{\circ}\text{C}$  for Chlamydia and  $37^{\circ}\text{C}$  for pili. After washing 5 times with a wash solution of 0.85% sodium chloride solution containing 0.05% Tween 20, the plate was tapped dry. A total of 100  $\mu\text{L}$  of test serum diluted 1 : 100, in EIA (phosphate buffer

0.15 M, pH 7.2 containing 0.8% skim milk powder and 0.05% Tween 20), was added to each well of the antigen coated plate and was incubated 30 °C for 2 hours. After washing 100 µL of anti-immunoglobulin was added. For the determination of IgG and IgA antibodies, the conjugates used were peroxidase conjugated, heavy chain specific, rabbit anti-human IgG or IgA (Dakopatts, Denmark) at a dilution of 1 : 4,000 and 1 : 2,000 in EIA diluent respectively. After being incubated at 37 °C for 2 hours and washing, 100 µL of the substrate was added.

The substrate was 0.15 M tetramethyl benzidine in 0.1 M sodium acetate (pH 6.0) containing 0.003% H<sub>2</sub>O<sub>2</sub>. Colour was permitted to develop at room temperature (20-25 °C) for 5 minutes for all antibodies except IgA antibody to Chlamydia, which required 30 minutes incubation. The reaction was stopped by the addition of 50 µL of 2 M H<sub>2</sub>SO<sub>4</sub> and the optical density (OD) at 450 nm was determined with a Flow Multiskan spectrophotometer.

Each sample was assayed in duplicate. Controls included positive and negative control sera and controls for nonspecific absorption. To evaluate the results, mean background OD was subtracted from the mean OD of each tested sample. This corrected OD was then used for the calculation of results.

### Statistical analysis

Statistical analysis was performed using the SPSS. Univariate analysis was done for categorical variables using the X<sup>2</sup> - test.

## Results

### Demographic characteristics

Table 1 summarizes the characteristics of the four different study groups and their husbands. The mean ages of infertile women with tubal or nontubal occlusion were similar (31.0 and 33.1 years), but were significantly different to the control population (26.7 years) or to the CSWs (22.9 years). There was no statistically significant

**Table 1.** Characteristics of the women studied and their husbands

	No.	Mean age year (± SD)	Duration of marital/intercourse year (± SD)	Age of first intercourse year (± SD)	History of STD (%)
1) pregnant women	68	26.7 (± 5.2)	3.6 ± 3.4	23.0 (± 4.3)	6 (8.8)
their husbands	68	30.7 (± 5.8)	-	-	33 (48.5)
2) infertile with tubal occlusion	60	31.0 (± 4.4)*	5.6 ± 3.3	23.3 (± 4.9)	9 (15.0)
their husbands	60	33.1 (± 4.8)	-	-	33 (55.0)
3) infertile with non-tubal occlusion	70	33.1 (± 3.8)*	5.2 ± 3.6	27.2 (± 4.5)*	7 (10.0)
their husbands	70	35.7 (± 5.7)	-	-	32 (45.7)
4) female CSWs	118	22.9 (± 4.1)*	2.4 ± 1.7	16.7 (± 2.3)*	99 (83.8)*

\* P < 0.05 compared with controls (pregnancy)

**Table 2.** Female and husband history of genital tract infection

Symptoms	Pregnancy N = 68	Tubal occlusion N = 60	Non-tubal occlusion N = 70	CSWs N = 118
Female history				
leukorrhoea	16 (23.5%)	16 (26.6%)	23 (32.8%)	48 (40.6%)*
PID	5 (7.4%)	11 (18.3%)	6 (8.5%)	58 (49.1%)*
abdominal pain				
with leukorrhoea	2 (2.9%)	5 (8.3%)	2 (2.8%)	19 (16.1%)*
Husband history				
dysuria	21 (30.8%)	17 (28.3%)	22 (31.4%)	
haematuria	4 (5.8%)	3 (5.0%)	6 (8.5%)	
urethral discharge	8 (11.7%)	3 (5.0%)	3 (4.2%)	

\* P < 0.05 compared with controls (pregnancy)

**Table 3.** Percentage of seropositive for chlamydial (L1), gonococcal pili antibody in various groups

Groups	No. of cases	No. of positive chlamydial antibody (%)		No. of positive pili antibody (%)	
		IgG	IgA	IgG	IgA
1. pregnant women	68	30 (44.1)	27 (39.7)	25 (36.7)	15 (22.0)
husbands	68	37 (54.4)	29 (42.6)	28 (41.1)	19 (27.9)
2. tubal occlusion	60	39 (65.0)**	22 (36.6)	34 (56.6)*	19 (31.6)
husbands	60	35 (58.3)	15 (25.0)*	21 (35.0)	12 (20.0)
3. non-tubal occlusion	70	26 (37.1)	35 (50.0)	17 (24.2)	11 (15.7)
husbands	70	28 (40.0)	38 (54.2)	22 (31.4)	15 (21.4)
4. female CSWs	118	58 (49.1)	7 (5.9)**	96 (81.3)**	72 (61.0)**

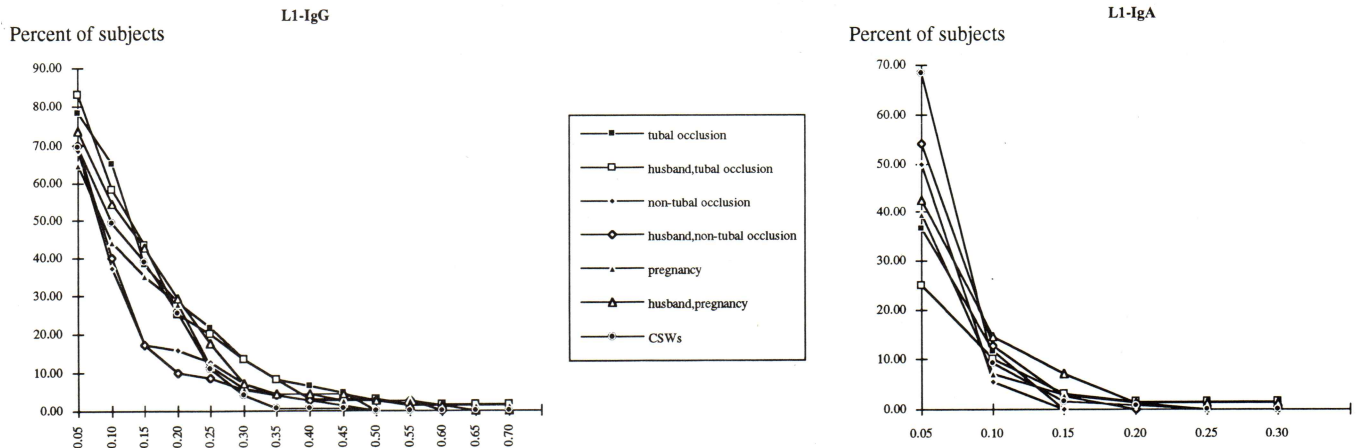
\* P < 0.05 compared with controls (pregnancy)

\*\* P < 0.01 compared with controls (pregnancy)

difference in the mean ages of the male partners in any of these groups.

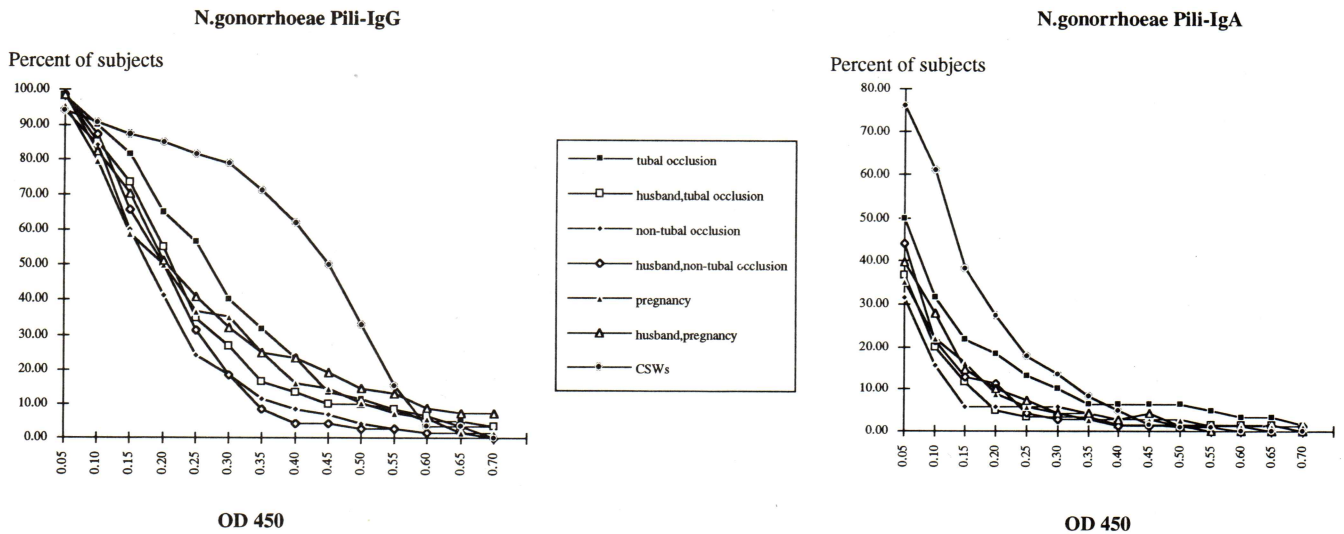
Of the 68 pregnant women, 21 (30.8%) had early sexual intercourse (less than 21 years old) when compared with 21/60 (35.0%) of the infertile women with tubal occlusion, 5/70 (7.1%) of the

infertile women with non-tubal occlusion, and 110/118 (93.2%) of the female CSWs. Of the 68 pregnant women, 4 (5.8%) had multiple sexual partners when compared with 10/60 (16.6%) of the infertile women with tubal occlusion (P < 0.05), and 2/70 (2.8%) of the infertile women with non-



**Fig. 1.** Graphs showing the influence of ELISA cut off on the prevalence of chlamydial (L1) antibody. Cut-off selected were 0.10 (L1-IgG) and 0.05 (L1-IgA).

(\*corrected OD = optical density of tested sample-optical density of controls)



**Fig. 2.** Graphs showing the influence of ELISA cut off on the prevalence of gonococcal pili antibody. Cut-off selected were 0.25 (Pili-IgG) and 0.1 (Pili-IgA).

(\*corrected OD = optical density of tested sample-optical density of controls)

tubal occlusion ( $P > 0.05$ ). Nearly half of the female CSWs (46.6%) had 5 or more sexual partners per week.

Table 2 shows the history of genital tract infection in the four study groups and their husbands respectively. One or more episodes of verified or suspected PID, leukorrhoea, and abdo-

minal pain with leukorrhoea in the females were reported ; including dysuria, haematuria, and urethral discharge in their husbands. A history of prior PID was found 11/60 (18.3%) in tubal occlusion group ( $P > 0.05$ ), and 6/70 (8.5%) in the non-tubal occlusion group ( $P > 0.05$ ), compared with 5/68 (7.4%) in the pregnant group.

Each tested sample was simultaneously assayed for IgG chlamydial/pili antibodies and IgA chlamydial/pili antibodies. The corrected OD determined in tested samples obtained from the variety of groups is shown in Figure 1 and Figure 2. The ELISA cut-off providing the best discrimination between the study groups was used to calculate the prevalence of chlamydial and gonococcal antibodies. The results at cut-off optical densities of 0.1/0.25 for IgG chlamydial/pili antibodies and 0.05/0.1 for IgA chlamydial/pili antibodies are shown in Table 3. By using the selected cut-off levels, positive results were demonstrated.

## Discussion

Serology is essential for assessing the role of *C. trachomatis* and *N.gonorrhoeae* in infertility because it is unlikely that patients will still be demonstrated infective with the organism that caused the original salpingitis by the time their infertility becomes apparent. Serum IgG antibodies, being long-lived, reflect the cumulative history of past exposure of the patients to infection and are therefore useful in epidemiological studies. Numerous studies have confirmed the increased prevalence of antibody to *C. trachomatis* in the sera of infertile women with tubal obstruction compared to such women without tubal obstruction or to fertile women. Between 40 and 75% of infertile women with tubal obstruction have serological evidence of past chlamydial infection.<sup>(12-15)</sup>

In this study, IgG antibodies to chlamydiae and gonococcal pili were significantly more prevalent ( $P < 0.01$  and  $P < 0.05$ ) in infertile women with tubal occlusion (65.0 and 56.6%) compared with the pregnant women (44.1 and 36.7%). We concluded that both *C. trachomatis* and *N. gonorrhoeae* are important causes of tubal pathology in Thai women. Women with tubal

infertility had a higher prevalence of IgG antibody to *C. trachomatis* than to *N. gonorrhoeae*. The presence of chlamydial antibodies was a more accurate predictor of tubal occlusion than other historical risk factors thought to cause permanent tubal damage.

In contrast, the measurement of IgA antibody to chlamydiae was significantly less prevalent ( $P < 0.05$ ) in the husbands of infertile women with tubal occlusion (25.0%) compared with the husbands of pregnant women (42.6%). This implies that the husbands of pregnant women had antibody to current rather than to past chlamydial infection, due to the variants of the study.

IgA antibodies have reported to be of particular importance in the serological diagnosis of acute chlamydial infection.<sup>(16)</sup> The determination of current chlamydial infection will be effective in predicting "at risk" individuals among gynaecologic patients before they develop tubal dysfunction. The long courses of antibiotics needed to treat genital chlamydial infection should indicate the prospects of controlling infertility due to *C. trachomatis*.

IgA antibody to chlamydiae was highly significant less prevalent ( $P < 0.0001$ ) in the female CSWs (5.9%). This suggests that the female CSWs in our region had low prevalence of antibody to current chlamydial infection. In contrast, IgG and IgA antibody gonococci were significantly more prevalent ( $P < 0.0001$  and  $P < 0.0001$ ) in the female CSWs (81.3 and 61.0%) compared with the pregnant women (36.7 and 22.0 %).

Determination of antibody to *C. trachomatis* and *N. gonorrhoeae* appears to be clinically useful in evaluating women with infertility. Our suggestion is that patients with the positive serological test most likely have tubal disease and are candidates for directly proceeding to laparoscopic examination,

therefore bypassing hysterosalpingography.

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