

# DETECTION OF CHLAMYDIAL AND GONOCOCCAL ANTIBODIES IN WOMEN WITH PELVIC INFLAMMATORY DISEASE AND INFERTILITY—AN AID TO DIAGNOSIS

By

R. A. BHUJWALA, T. D. BISWAS, V. BHARGAVA, N. DANG AND R. CHAUDHRY

## SUMMARY

A total of 65 patients of Pelvic Inflammatory Disease and Infertility were studied for the presence of antibodies against *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. Indirect immunoperoxidase test (Ipazyme kit) and ELISA test were used for detection of chlamydial and gonococcal antibodies respectively. 62.85% of PID and 60% of Infertility cases were found to be positive for chlamydial antibodies. Only 11.4% and 3.3% of the PID and infertility cases had gonococcal antibodies. Antibody detection is a sensitive, specific and noninvasive test for diagnosing Pelvic Inflammatory Disease and Infertility.

### Introduction

*Chlamydia trachomatis* is an obligate intracellular parasite affecting the urogenital system of human beings. The genital site most commonly affected in the female is the cervix (Schachter *et al* 1975). From the cervix the infection can extend to the fallopian tubes causing pelvic inflammatory disease (PID) which includes inflammation of the cervix, uterus, fallopian tubes and ovaries (Schachter and Dawson, 1978) subsequently leading to infertility (Kane *et al* 1984). About 22% to 58% cases suffering from *C. trachomatis* cervical infection develop PID as a result of which 11%-35% become infertile (Kane

*et al* 1984). Another cause of PID and infertility in women is gonorrhoea. According to Bowie and Jones (1981) in 10% cases of PID, *N. gonorrhoeae* is the responsible agent. The diagnosis of chlamydial and gonococcal PID and infertility based on isolation of the organism is not easy due to the difficulty in obtaining the samples from the specific sites like inflamed or occluded fallopian tubes. On the other hand, detection of antibodies to *C. trachomatis* and *N. gonorrhoeae* is easier as it involves only collection of patient's blood. In PID and infertility presence of antibodies indicates infection with these organisms (Schachter *et al* 1978). As PID is a major problem in India, and no work has been done on the antibody levels of *C. trachomatis* and *N. gonorrhoeae* in this infection a small study has been conducted with the Ipazyme kit (Savyon Diagnostic Ltd.) to detect IgG and IgA

From: Department of Microbiology\*, Obstetrics and Gynaecology\*\*. All India Institute of Medical Sciences, Ansari Nagar, New Delhi-110 029.

Accepted for publication on 22-9-87.

antibodies to *C. trachomatis* in women clinically suspected to be suffering from PID and infertility. The IgG antibodies to *N. gonorrhoeae* have been tested by the ELISA test (Bhujwala *et al* under publication).

#### Material and Methods

i. Study group: This comprised of the following.

35 patients suffering from PID (i.e. fever, abdominal pain, pelvic tenderness, pelvic mass, peritoneal involvement).

30 women with primary infertility.

5 Healthy controls.

ii. Clinical sample: Blood was collected on a one time basis from these patients on the day they reported to the hospital and serum was separated and stored at 2°-8°C.

iii. Test procedure: All the samples were subjected to indirect immunoperoxidase assay as described in the Ipazyme kit by Savyon Diagnostics Ltd. for detection of IgG and IgA chlamydial antibodies. Results were interpreted as per Table I.

iv. All sera were also tested for gonococcal antibodies by the ELISA test (Bhujwala *et al* under publication) using

commercially available *N. gonorrhoeae* antigen, anti human IgG horse radish peroxidase conjugate and O-phenylene diamine with hydrogen peroxide substrate.

#### Results

The results of the study are given in Table II. It is seen that 62.85% of cases suffering from PID were positive for *C. trachomatis*. Similarly, 60% of infertile women were suffering from chlamydial infection as indicated by the antibody titre. The statistical analysis carried out on PID and infertility cases suffering from chlamydial infection versus controls revealed a statistically significant difference in the antibody titres of PID and infertility versus controls ( $p < 0.5$ ). Elisa test for gonococcal antibodies revealed that only 4 (11.4%) of the 35 patients suffering from PID and 1 (3.3%) of the 30 patients suffering from infertility had gonococcal antibodies. All these 5 patients had concurrent chlamydial infection (Table II).

#### Discussion

The laboratory diagnosis of *N. gonorrhoeae* can be handled by most laboratories but that of *C. trachomatis* needs

TABLE I  
Interpretation of Results by Ipazyme Test

Assay	Dilution	Possible results							
		1	2	3	4	5	6	7	8
IgG	1:64	+	+	+	+	+	+	-	-
IgG	1:128	+	+	-	-	±	-	-	-
IgA	1:16	+	-	+	±	-	-	+	-

1,2 and 3 = active infection  
4 = Borderline of active infection  
5 and 6 = Positive  
7 = Rare possibility, repeat test  
8 = Negative

TABLE II  
Chlamydial and Gonococcal Antibodies by Ipazyme ABD Elisa Test

Disease	Nos. studied	Chlamydial infection			Gonococcal antibodies
		Active infection	Positive	Negative	
PID	35	15 (42.85)	7 (20.0)	13 (37.14)	4 (11.4)
Infer- tility	30	8 (26.66)	10 (33.33)	12 (40.00)	1 (3.3)
Controls	5	0	0	5	0

N.B. Figures within parenthesis indicate percentage.

Chlamydial infection, PID vs. control— $X^2$  value 4.67, Significance  $P < 0.05$ .

Chlamydial infection, Infertility vs. control— $X^2$  value 4.00, Significance  $P < 0.05$ .

culture in McCoy or Hela cell line. The procedure requires a complete set up of tissue culture facility which is not available in most laboratories in India. Secondly to obtain clinical material from the actual site of infection in cases of PID and infertility is difficult, as the material has to be collected from the cul-de-sac or by needle aspiration of fallopian tubes or from the affected site at the time of surgery. Such invasive procedures are not appreciated by clinicians, specially in the acute infections. On the other hand, elevated titres of IgG and IgA chlamydial and gonococcal antibodies serve as markers for early detection of active infection, with these organisms (Moore *et al* 1982, and Jones *et al* 1982). Elevated anti *C. trachomatis* IgG antibody titres are specially found in salpingitis and infertility (Moore *et al* 1982, Jones *et al* 1982 and Piura *et al* 1985). Hence it was decided to determine the antibodies of *C. trachomatis* and *N. gonorrhoeae* in a small number of patients suffering from these infections. It is seen that 62.85% of the cases suffering from PID had antibodies to *C. trachomatis*, while only 11.4% had antibodies to *N. gonorrhoeae*. Of the 62.85% patients with antibodies to *C. tracho-*

*matis*, 42.85% had active infection with IgG titres varying between 1:64 to 1:128 and IgA being either present or absent. Another 20% of PID cases were positive for chlamydial antibodies with IgG titres of 1:64 without IgA antibodies. Thus two thirds of the PID cases with chlamydial antibodies were suffering from active infection. According to Mardh *et al* (1981) antichlamydial antibody titres correlate with the severity of the tubal inflammation and the duration of lower abdominal pain. Similar results have been reported by Wlner-Hanssen and Westrom (1983) according to whom 84.6% of PID patients had serological/cultural evidence of *C. trachomatis*. Ripa *et al* (1980) in a study conducted on 206 patients of salpingitis showed that 118 (57.2%) had chlamydial IgG antibodies in titres of 1:64 or more and in 80 paired sera, 28 (35%) showed rising titre of antibodies.

In cases of infertility 26.66% in the present study had active infection and another 33.33% had chlamydial antibodies Kane *et al* (1984) laproscopically studied the incidence of *C. trachomatis* antibodies in infertile women varified to have peripheral tubal disease. The incidence was found to be 35.7%. Con-

current infection with *C. trachomatis* and *N. gonorrhoeae* have been reported in 23% cases of PID (Wlner Hanssen and Westrom 1983). In this study 11.4% patients suffering from PID had concurrent infection with *C. trachomatis* and *N. gonorrhoeae* and another 3.3% cases of infertility also had antibodies to both these organisms.

As none of the controls showed the presence of these antibodies it was assumed that Ipazyme test was a specific test for the detection of chlamydial antibodies.

The results of this study indicate that in majority (62.85%) of cases of PID, *C. trachomatis* is the probable causative agent whereas *N. gonorrhoeae* is responsible for only 11% cases. Similarly, in infertility 60% had chlamydial antibodies whereas only 3% had gonococcal antibodies. For the diagnosis of chlamydial antibodies the Ipazyme test is a sensitive, easy to perform test and can be employed by peripheral laboratories specially to detect cases of PID which is a major problem in India. The availability of serologic markers for *C. trachomatis* and *N. gonorrhoea* infection reduces the necessity for invasive procedure in diagnosis and as these diseases can be effectively treated with tetracycline and erythromycin the tests can be employed for early detection of these infections, so that the timely treatment can prevent the disease from damaging the upper genital tract.

#### References

1. Bhujwala, R. A., Biswas, T. D., Kumar, R., Bhargava, N. C., Bhargava, V. and Shrinivas: Serological diagnosis of gonorrhoea by Enzyme Linked Immuno Sorbent Assay (ELISA)—under publication. *Indian J. Med. Res.*
2. Bowie, W. R. and Jones, H.: Acute Pelvic inflammatory disease in out patients. Association with chlamydia trachomatis and *N. gonorrhoeae*. *Ann. Int. Med.* 95: 685, 1981.
3. Jones, R. B., Ardery, B. R., Hui, S. L. and Cleary, R. E.: Correlation between serum antichlamydial antibodies and tubal factor as a cause of infertility. *Fertil. Steril.* 38: 553, 1982.
4. Kane, J. L., Woodland, R. N., Forey, T., Darougar, S. and Eldar, M. G.: Evidence of chlamydial infection in infertile women with and without fallopian tube obstruction. *Fertil. Steril.* 42: 843, 1984.
5. Mardh, P. A.: An overview of infectious agents of salpingitis their biology and recent advances in methods of detection. *Am. J. Obstet. Gynec.* 138: 933, 1980.
6. Mardh, P. A., Lind, I., Svensson, L., Westrom, L. and Moler B. R.: Chlamydia trachomatis *Mycoplasma hominis* and *Neisseria gonorrhoeae* in sera from patients with acute salpingitis. *Br. J. Vener. Dis.* 57: 125, 1981.
7. Moore, D. E., Foy, H. M., Daling, J. R., Gryston, J. T., Spadoni, L. R., Wang, S. P., Kuo, C. C. and Eschenbach, D. A.: Increased frequency of serum antibodies to *C. trachomatis* in infertility due to distal tubal disease. *Lancet*, 2: 574, 1982.
8. Piura, B., Sarov, T., Sarov, B., Kleinman, D. Claim, W. and Insler, V.: Serum IgG and IgA antibodies specific for chlamydia trachomatis in salpingitis patients as determined by the immunoperoxidase assay. *Eur. J. Epidemiol.* 1: 110, 1985.
9. Ripa, K. T., Svensson, L., Treharne, J. D., Westrom, L. and Mardh, P. A.: *C. trachomatis* infection in patients with laparoscopically verified acute salpingitis. *Am. J. Obstet. Gynec.* 138: 960, 1980.
10. Schachter, J., Hill, E. C., King, E. B., Coleman, V. R., Jones, P. and Meyer, K. F.: Chlamydial infection in women with cervical dysplasia. *Am. J. Obstet. Gynec.* 123: 753, 1975.
11. Schachter, J. and Dawson, C. R.: Human chlamydial infection PSG Publishing Co., Inc. Littleton, M. A., 1978.
12. Wlner Hanssen, P. and Westrom, L.: Second look laparoscopy after acute salpingitis. *Obstet. Gynec.* 61: 702, 1983.