ORIGINAL ARTICLE

The Prevalence of *Chlamydia Trachomatis* in Patients with Pelvic Inflammatory Disease

J Ravindran, MRCOG*, Y I Tan, MRCOG*, Y F Ngeow, FRCPath**, *Department of O&G, Hospital Seremban, 70300 Seremban, Malaysia, **Department of Medical Microbiology, University of Malaya, 50603 Kuala Lumpur, Malaysia

Summary

Chlamydia trachomatis is recognized as the most prevalent sexually transmitted organism in many parts of the world. Most complications associated with chlamydial infection in women and their infants can be avoided by appropriate treatment. However, treatment is often not initiated because infections are frequently asymptomatic. The identification of at risk patients and treatment of these patients is a practical clinical approach in the reduction of transmission and prevention of complications.

The prevalence of chlamydial infection among patients with pelvic inflammatory disease admitted to Seremban General Hospital was 22.7%. The difference in seropositivity between PID patients (20.5%) and antenatal controls (2.3%) was statistically significant. The corresponding cervical antigen detection rates were 6.8% and 2.3% respectively. Chlamydial infection should be screened for in gynaecological patients and antibiotic policies should take cognizance of the aetiological role played by this organism in pelvic inflammatory disease.

Key Words: Chlamydia trachomatis, Pelvic inflammatory disease

Introduction

Interest in genital chlamydial infection has intensified in the last two decades after more than 80 years since Halberstaedter and von Prowazek demonstrated inclusion bodies in the conjunctivae of infants with neonatal conjunctivitis and in the cervices of their mothers more than 80 years ago[1]. *Chlamydia trachomatis* and its role in causing pelvic inflammatory disease (PID) was first reported by Mardh *et al* in 1977[2]. This organism is now recognised as the most common identifiable sexually transmitted organism in developed countries[3].

Most complications associated with chlamydial infection in women and their infants could be avoided by appropriate treatment. However, treatment is often not initiated as infections are frequently asymptomatic and general screening is not cost effective. The identification of risk characteristics for chlamydial infections in women has made selective testing a practical clinical approach.

In recent years, newer and simpler techniques to detect chlamydial antigen and antibody have been made available. This study was initiated in the state of Negeri Sembilan in Malaysia with the aim of finding out the prevalence of chlamydial infections in patients presenting with pelvic inflammatory disease (PID) in Seremban General Hospital, an 800-bed referral hospital.

Materials and Methods

This study was a prospective study from 1st November 1993 to 30th October 1994. Patients identified as having PID in the gynaecological ward and gynaecological outpatient clinic at the Seremban General Hospital were enrolled into the study. Asymptomatic antenatal mothers of similar age, race

and social class attending the same hospital over the same period served as controls.

The criteria for the selection of PID patients for the study were the presence of lower abdominal pain, vaginal discharge, cervical tenderness, adnexal tenderness or uterine tenderness. Informed consent was obtained from each patient after the purpose and the content of the study were explained to her. Protocol questionnaires covering known risk factors and symptoms of PID were filled. The patients were examined and endocervical swabs for chlamydial antigen detection and gonococcal isolation were then taken. Blood specimens were drawn for chlamydial serology at the same time.

Sampling procedure:

A) Endocervical specimen
From each study subject, two swabs were collected from the endocervix, first for gonococcal isolation then for chlamydial antigen detection. The gonococcal swab was Gram-stained for intracellular Gram-negative diplococci and directly inoculated onto Thayer-Martin agar. Culture plates were incubated for up to 96 hours in a candle extinction jar at 37°C and then examined visually for the presence of colonies of *Neisseria gonorrhoeae* which were further identified by Gram staining and oxidase testing.

The STD-EZE Sample Collection and Transport Kit for chlamydia (Abbott Laboratories, Chicago, USA) was used in the following manner - excess mucus was removed from the endocervix with a cotton tipped swab. A STD-EZE swab was then rotated inside the endocervical canal for 15 to 30 seconds to ensure adequate sampling. The swab was then placed in the STD-EZE transport tube and transported to a single reference laboratory where the Chlamydiazyme test (Abbott Laboratories), an enzyme immunoassay (EIA) using a labelled monoclonal antibody to detect chlamydial antigen, was carried out within 7 days of specimen collection following the manufacturer's instructions. All positive samples in the Chlamydiazyme test were retested with a confirmatory reagent. Only confirmed positive results were included in the final analysis.

B) Serology
Five ml of blood were taken at the same time for chlamydial serology. Blood samples were centrifuged and the sera extracted kept at - 20° C for batch analysis. Sera were tested for the presence of chlamydial IgG, IgM and IgA by a microimmunofluorescence technique (MIF) using *C. trachomatis, C. pneumoniae* and *C. psittaci* antigens obtained from the Washington Research Foundation, Seattle, USA and the Johns Hopkins University, Baltimore, USA. Serum dilutions were made from 1:8 to 1:512 for IgG and from 1:8 to 1:32 for IgM and IgA. Samples for IgM testing were first treated with anti-human IgG to inactivate chlamydial IgG which might give rise to false negative IgM results.

Results

A total of 88 patients participated in the study, 44 in the PID study group and 44 antenatal patients recruited as controls.

Chlamydial Infection

Ten patients (22.7 %) in the study group were found to have recent chlamydial infection. In these 10 patients, seven had chlamydial IgG titres of 1:64 to ≥1:512 (five with IgM titres ≥1:32, two with IgA 1:16 as well), two were positive for both antigen and antibody (IgG 1:256, IgM 1:16 and IgG 1:16, IgM≥1:32) and one had chlamydial antigen without raised antibodies.

In the control group, two patients (4.5 %) had evidence of chlamydial infection. One had a raised antibody titre (IgG 1:128) indicating infection at an undetermined time and the other was positive for antigen with an IgG titre of 1:32. Both antigen and antibody detection rates were higher for the PID patients (Table I) but only the antibody detection rates were significantly different ($P<0.05$).

Gonococcal Infection

In the PID group, one patient (2.3 %) was found to have gonococcal infection co-existing with chlamydial infection. This patient was a 17 year-old single woman who had multiple sexual partners. No other patient from the study or the control groups was found to have gonococcal infection.

Table I
Positive chlamydial antigen and antibody tests in PID patients and controls

Patient	Test	No. Tested	No. Positive
PID	Antigen	44	3 (6.8%)
	Antibody *	44	9 (20.5%)
Controls	Antigen	44	1 (2.3%)
	Antibody *	44	1 (2.3%)

* *A positive titre was taken to be IgG > 1 : 64 or IgM or IgA >1:16 in the presence of IgG*

Patients' Profile

1) Ethnic Group
The majority of the patients (88.6%) who presented with PID were Malays and Indians. This is a reflection of the ethnic distribution among patients seeking treatment in Seremban General Hospital as many Chinese patients seek treatment from private gynaecologists. Chlamydial infection was found in 31.6% of the Malay patients who presented with PID, as compared to 15% of the Indian and 20% of the Chinese PID patients.

2) Age
Most of the PID patients were within the 20-40 year age group (88.6%). Only three (6.8%) were under 20 years old. Similarly 70% of the chlamydia-positive PID patients were in the 20-40 year age group but two (20%) were under 20 years old.

3) Social Class
Twenty-eight patients (63.6%) who participated in the study were in the lower social classes IV and V. Although chlamydial infections were found in all the five social classes, the majority (60%) of the chlamydia positive patients were from the lower social classes IV and V.

4) Marital Status
Forty of the PID patients and 7 out of the 10 patients positive for chlamydia were married. The only 2 widows and one of the two single women in the study group were chlamydia-positive.

5) Subfertility
Twenty patients (45.5%) had subfertility. Nine (20%) were unsuccessful in conceiving in a period of more than 2 years before entry into the study. Similarly, in the PID patients in whom chlamydial infections were identified, 50% complained of subfertility and 20% were unable to conceive in more than 2 years before entry into the study.

6) Patients' sexual activity
Forty-two of the 44 patients were still sexually active at the time of entry into the study. The only 2 widows in the study claimed to be non sexually active but both were chlamydia positive.

7) Status of the male sexual partner
Forty-one (93.2%) of the PID patients and 60% of chlamydia-positive patients claimed that their sexual partners were asymptomatic and had no history of sexually transmitted diseases. Except for 2 chlamydia-positive patients, all the patients' partners had monogamous marriages.

8) History of contraception
Twenty-six patients (59.1%) practised no contraception. Six (23.1%) of these were chlamydia positive. In the remaining 18 patients who were not on contraception, 4 (22.2%) were chlamydia-positive including 3 of 7 patients (15.9%) who used intrauterine contraceptive devices (IUCD) and 1 of 2 patients who used condoms.

Symptoms

In addition to the symptoms criteria for inclusion in the study, other symptoms such as pyrexia, pruritus vulvae, malodorous vaginal discharge and dyspareunia were also looked into.

The majority of the PID patients (61.4%) and chlamydia positive patients had no fever, about 50% of them had pruritus vulvae and 54.5% of the PID patients and 60% of patients with positive chlamydial infection did not have malodorous vaginal discharge. In contrast to the PID patients, 72.7% of whom had dysparaeunia, 80% of the patients with positive chlamydial infection did not complain of dysparaeunia.

Laparoscopic Examination

In view of limited facilities, laparoscopic examination was carried out only when there was failure to respond to treatment or as part of the investigation of infertility when the infection had settled. Hence only 15 patients (34%) had laparoscopic examination in this study. In all 15 patients, there was laparoscopic evidence of current or past PID. Tubal blockage was observed in two of five patients who were positive for chlamydial infection.

Discussion

Chlamydial infection has increasingly become recognised as a cause of acute salpingitis, chronic pelvic inflammatory disease and infertility. It is now estimated with the use of serologic and culture methods that approximately 50 % of cases of acute salpingitis are chlamydia related[4,5] and that the incidence of post infectious complications of chlamydia may exceed those of gonorrhoea[6].

In tropical countries, there is generally a lack of accurate data on the prevalence of chlamydial infections as facilities for the isolation of *C trachomatis* are rather limited. Several epidemiological surveys have indicated that chlamydial genital tract infections in developing countries may be as common as or more common than those occurring in developed countries[7,8]. In this light, this study was designed to determine the prevalence of *C trachomatis* infections in patients presenting with pelvic inflammatory disease in Seremban General Hospital.

The overall prevalence of chlamydial infection among PID patients in this study was 22.7 % (20.5% by serology and 6.8% by antigen detection). The prevalence of significantly raised antibody titres is comparable to that (20-25%) reported by Mardh P.A. for European patients[9] but the cervical antigen detection rate is much lower than the 27.6% detected among Malaysian commercial sex workers with PID[10]. Both cervical antigen and serum antibody are not direct evidence of PID. Cervical specimens are often chlamydial negative in patients with chlamydial salpingitis or endometritis and raised serum antibodies could indicate infection in any part of the body other than the endometrium or the Fallopian tubes. It is generally accepted, however, that women showing significant chlamydial antibody levels with negative cervical tests are likely to be having chlamydial upper genital tract infection. In these women, further evidence of chlamydial infection can sometimes be obtained by the detection of the organism in urethral or anorectal specimens from the patient or her sexual partner.

Compared to commercial sex workers, the lower cervical chlamydial detection rate among PID patients in this study could be a reflection of the lower exposure rate among the general female population but could also be due to the majority of patients having been treated by their general practitioners before referral to the Seremban General Hospital.

In published data, the percentage of coexistent chlamydial and gonococcal infection ranges from 32% to 62%[11,12,13,14]. In this study only one patient was found to have gonococcal infection co-existing with chlamydial infection. As in other reports *C. trachomatis* was found surpassing *N. gonorrhoeae* as the cause of PID in women[2,3,11,14,15,16].

Oral contraceptive pill usage has been associated with a decreased risk of acute salpingitis[17] but an increased risk of chlamydial cervical infection due in part to the increased ectopy of the cervix[18]. In this study, 60% of chlamydia-positive patients had no history of contraceptive use; 30 % used IUCD. None of the 6 PID patients who had used oral contraceptives had chlamydial infection. On the other hand 3 of 7 PID patients who used IUCD were infected by chlamydia, suggesting that IUCD use predisposes to chlamydial infection. Overall, however, there was no difference in the chlamydial infection rate between those on contraception (23.1%) and those not on contraception (22.2%).

Studies in which pregnant mothers were screened for the presence of *C trachomatis* have shown that maternal infection rates vary according to socio-economic background and range from 2 to 37%[19,20]. The antigen detection rate of 2.3% in this study is similar to those found in previous studies in Malaysia[10]. The effect of cervical infection by *C trachomatis* on pregnancy

outcome has been the subject of many investigations but still remains unclear.

For the diagnosis of chlamydial PID, selection of the most appropriate laboratory test depends on the availability of the test, frequency of use, logistics of specimen transport and the prevalence of chlamydia in the population tested. Until recently, cell culture was the only method available for a reliable diagnostic test. This is highly specific and sensitive but technically cumbersome and expensive. Most importantly, successful culture depends on the delivery of viable organisms to the laboratory at 4°C within 24 hours[21]. In view of its difficult technique and high cost, cell culture is not available for the detection of *C trachomatis* in Seremban General Hospital.

Advances in technology have provided alternatives for chlamydia detection which are quicker and cheaper than cell culture. Antigen detection tests like enzyme immunoassays and direct immunofluorescence using labelled monoclonal antibodies to the chlamydial major outer membrane protein have been widely used. The EIA has the advantage of eliminating subjectivity as the test endpoint is measured objectively by spectrophotometry and samples are usually batch-tested, making EIA suitable for high volume testing programmes. Most commercially available chlamydial EIAs have been shown to be of adequate sensitivity and specificity when compared with cell culture. The Chlamydiazyme test was reported to have a sensitivity of 85% and specificity of 99.2%[22].

A variety of tests are available for the detection of antibodies to chlamydiae. These tests may provide evidence of infection although there is still considerable confusion over the interpretation of serological test results for the diagnosis of individual cases of chlamydial infection[23]. Treharne *et al* were the first to associate *C trachomatis* IgG (MIF) antibody titres of 1: 64 or more with the diagnosis of salpingitis and tubal infertility[24]. Other studies correlating antibody titres with isolation rates also showed that a IgG titre of 1: 64 in women with cervicitis may indicate current chlamydial infection[25]. Hence antibody titres of 1: 64 or more were taken as positive for chlamydial infection in this study.

It has been shown that immune responses may interfere with recovery of the organism and that *C trachomatis* isolation declined with increasing antibody titre[2]. This is illustrated in this study by the fact that the 3 antigen-positive PID patients had lower IgG titres of <1:8, 1:16 and 1:256 whilst 5 of the 7 antigen-negative patients had IgG titres ≥1:512.

Conclusion

C trachomatis was found to be a causative agent of pelvic inflammatory disease in the local population. In view of the serious complications which are associated with chlamydial infection such as infertility, chronic pelvic pain, pelvic sepsis and neonatal pneumonia and the high cost of treating these complications, an effort should be made to screen at risk patients. Antibiotics that eradicate *C trachomatis* should be given to patients who are infected with this organism. It is appropriate to treat PID patients who have negative endocervical tests for chlamydia but show seroconversion or have significantly raised chlamydial antibodies.

It is hoped that with the advancement of diagnostic techniques, a rapid on-site antigen detection test could be made freely available at a low cost for the initial screening of all women attending gynaecological clinics and antenatal mothers in an effort to eradicate this subtle pathogen.

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