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Detection of Chlamydia trachomatis by the Polymerase Chain Reaction in the Cervices of Women with Pelvic Inflammatory Disease

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ABSTRACT

Objective 1. To determine the clinical features and the prevalence of Chlamydia trachomatis in women with pelvic inflammatory disease, and 2. To assess the association between intrauterine contraceptive device use and the prevalence of Chlamydia trachomatis.

Design A prospective, observational study.

Setting Guangzhou Maternal and Neonatal Hospital, Guangdong, China.

Subjects and methods Endocervical samples from 85 women with pelvic inflammatory disease were tested for Chlamydia trachomatis, by the polymerase chain reaction and enzyme immunoassay.

Results Eighty-five patients with pelvic inflammatory disease were verified at laparoscopy or pathology. Thirty women had evidence of pelvic adhesions. Chlamydia trachomatis was detected in the cervices of women with pelvic inflammatory disease in 45 of 85 patients (52.9%) by the polymerase chain reaction, 27 of 78 (34.6%) by the enzyme immunoassay. Only 15 of these women (19.2%), of whom had positive results when tested with the polymerase chain reaction, had enzyme immunoassay that were positive for Chlamydia trachomatis. Chlamydia trachomatis was identified in the genital tract in 22 of 45 women (48.9%) with intrauterine contraceptive devices and 23 of 45 women (51.1%) not using contraceptive methods ($P > 0.05$).

Conclusion The polymerase chain reaction appeared to be more sensitive than enzyme immunoassay in detecting Chlamydia trachomatis in the cervices of women with pelvic inflammatory disease. This study suggests that early laparoscopy in hospitalised women improves diagnostic precision and provides information for future fertility. Our findings support that chlamydia-infected intrauterine contraceptive device users are not at increased risk for pelvic inflammatory disease.

Key words : Chlamydia trachomatis, pelvic inflammatory disease, polymerase chain reaction, enzyme immunoassay, intrauterine contraceptive devices

Chlamydia trachomatis is the most common cause of pelvic inflammatory disease (PID) in the Western world.[1] During the last decade, in the developing world the number of new cases of uncomplicated sexually transmitted genital tract infections reported in women has continued to increase whilst at the same time there has been a parallel rise in the prevalence of acute salpingitis. The number of new cases of chlamydial genital tract infections have increased. In one study of African women with ectopic pregnancies, 47% had antibodies to the gonococcus and 31% to chlamydia.[2] Such that Chlamydia trachomatis may now be the major aetiological agent of pelvic inflammatory disease (PID) in Southern China. Use of the polymerase chain reaction technique to detect C. trachomatis offer a significant advance over other methods.

The aim of our study was to determine the demographic features and the clinical and laparoscopic findings in a cohort of women from urban population presenting with a clinical diagnosis of PID and also to assess the association between intrauterine contraceptive device use and the prevalence of Chlamydia trachomatis.

Materials and Methods

Between May 1995 and May 1996, women with PID from the inner city area of Guangzhou were admitted to our hospital. The study population consisted of 85 women with PID, women eligible for the study were those complaining of lower abdominal pain and whose on examination had signs suggestive of an upper genital tract infection (i.e. cervical motion pain, uterine and adnexal tenderness) plus pyrexia around 38°C or an erythrocyte sedimentation rate greater than 15 mm/hr. The nature of symptoms (i.e. abdominal pain, prolonged, or irregular vaginal bleeding, abnormal vaginal discharge, urinary frequency) were recorded.

The study population with PID were diagnosed either by laparoscopy or pathology. The minimum requirement for the diagnosis of PID was a finding of tubal swelling, hyperemia, tubal exudate, tortuosity, loss of fimbrial anatomy, adhesions in the pelvic area or adnexal mass.

Systemic antimicrobial therapy was commenced in all cases of PID. Inpatient therapy was continued until a clinical cure was confirmed, based on an improvement in clinical condition and resolution of pelvic inflammation judged by bimanual pelvic examination. All cases were encouraged to return for follow up at 7 to 10 days. The patients were divided into two groups : 1. those with chlamydial infection associated PID and 2. those with non-chlamydial infection PID.

Cervical samples were obtained at the first day patients were admitted to the hospital. After cervical mucus was wiped away, two cotton swabs were inserted into the endocervix, rotated for 10 seconds.

Polymerase Chain Reaction (PCR) : Cervical samples were collected by immersing the endocervical swabs into sterile tubes containing 1.5 ml phosphate-buffered saline. The sample was agitated,transferred into a microcentrifuge tube and centrifuged at 10,000 rpm for 5 minutes. The pallet was then resuspended in 50 μl lysis buffer containing 0.45% NP40, 0.45% Tween 20 and 200 (μg/ml proteinase K. After incubated at 56°C for 1 hour and 95°C for 10 minutes, the sample was centrifuged again at 10,000 rpm for 5 minutes. 4 μl supernatant was used in each amplification. 46 μl agents mixture was added to yield a final reaction volume of 50 μl containing 10 mM Tris. HCl pH 9.0, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 10 pmol of each primer and 1.5 unit Taq DNA polymerase. The reactions were performed on a thermocycler as follows : 94°C 30 sec, 55°C 45sec, 72°C 60sec, 35 cycles ; 72°C 10 min. The amplified products were subjected to 1.5% agarose gel electrophoresis, and the DNA bands were viewed under ultra-violet light after staining with ethidium bromide. The presence of a 225 bp DNA band indicated the presence of C. trachomatis.

Enzyme Immunoassay : Endocervical swab specimens were also tested for Chlamydia trachomatis antigen using Clearview Chlamydia kit (Unipath Limited, England). Sample collection and test performance were conducted as the manufacturer described. The swab was incubated at 80°C for 10-12 minutes with 0.6 ml Extraction Buffer containing 0.1% sodium azide. Five drops of swab extract was added to the sample window of the Test Unit, and the result was read in the result window 15-60 minutes after addition of extract to the Test Unit. An integral control ensured proper performance of the test.

Results

Detection of C. trachomatis in cervical samples : C. trachomatis was detected by

Table 1. Clinical and laboratory findings in 85 women with PID

Variables	Chlamydial PID n = 45 n (%)	Non-chlamydial PID n = 40 n (%)	P-value
Infertility	8 (17.8)	9 (22.5)	> 0.05
IUD users	22 (48.9)	16 (40.0)	> 0.05
Nonusers	23 (51.1)	24 (60.0)	> 0.05
Urinary symptoms	12 (26.7)	8 (20.0)	> 0.05
Vaginal bleeding	18 (40.0)	7 (17.5)	< 0.025
Vaginal discharge	21 (46.7)	16 (40.0)	> 0.05
Pelvic mass	16 (35.6)	8 (20.0)	> 0.05
Temp (>38°C)	7 (15.6)	9 (22.5)	> 0.05
ESR (>15mm/h)	17 (37.8)	14 (35.0)	> 0.05

polymerase chain reaction in cervical samples from 45 of the 85 women (52.9%) with PID. When compared with using enzyme immunoassay method, 27 ot the 78 women (34.6%) with PID had C.trachomatis in the cervix, only 15 (19.2%) of the patients who had PID, had positive polymerase chain reaction results, also had positive enzyme immunoassay for C.trachomatis. The difference in chlamydia detection between the two assays was significant ($P < 0.02$).

Clinical features : The clinical features of the women studied are shown in Table 1. Twenty-five cases of the women with PID were irregular vaginal bleeding, but urinary symptoms were reported less frequently by women with PID ($n = 20$). The useful clinical sign that discriminated between the two groups was the finding of a mucopurulent vaginal discharge in the women with PID.

Table 1 shows the demographic and clinical features of the women with chlamydia-associated PID. Women with chlamydia PID (n=45) were younger (median age 31 years, range 21 to 41 years) than those with non-chlamydial disease (n=40, median age 34 years, range 22 to 60 years), and were more likely to give a history of irregular vaginal bleeding than women with non-chlamydial disease ($P<0.025$). Chlamydia-infected IUD users are not at increased risk for PID when compared with women not using a contraceptive method ($P>0.05$).

Laparoscspic findings : Eighty-five cases with PID were verified at laparoscopy or pathology. Eight cases of pelvic masses were managed by laparotomy and three tubo-ovarian abscesses were drained from the needle under ultrasonographic control.

Thirty women with PID had evidence of pelvic adhesions. Women with a history of infertility were more likely to have extensive adhesions and tubal occlusions at presentation. For the women with pelvic masses, the presence of adnexal mass was influenced by a previous history of acute salpingitis or longer duration of symptoms prior to presentation.

Discussion

There is overwhelming evidence to suggest that C. trachomatis play a major role in the pathogenesis of PID in the developed and developing countries. This series demonstrates the clinical, laparoscopic and chlamydial findings in a cohort of women from an urban population presenting with symptoms and signs of PID. It represents the largest number of laparoscopically verified cases of PID reported from Southern China to date. C. trachomatis was identified in the genital tract in 38.5% of the women with acute salpingitis.[3] In both Europe and North America, more recent investigations have generally found a higher proportion of C. trachomatis in women with symptoms of PID. Chlamydia has been isolated in as many as 51% of some North American populations.[4] Witkin reported that 9 of the 15 women (60%) with salpingitis had positive results when tested with the polymerase chain reaction for cervical C. trachomatis.[5]

Recent studies have further emphasized that polymerase chain reaction analysis of genital tract specimens from women with symptoms for the presence of C. trachomatis should lead to a greatly improved understanding of the role of this organism in genital tract infections. Our study suggested that C. trachomatis can be detected with an increased prevalence in the cervix by the polymerase chain reaction.

Laparoscopy is now used more frequently to diagnose PID in our hospital, but in general it is likely that the majority of women with the disease continue to be diagnosed and treated as

outpatients. So hospital data must under-estimate the incidence of PID in the community. Laparoscopic confirmation of PID not only avoids delay in specifically directed therapy, but it also allows an accurate assessment of disease severity. This provides important information regarding the infertility. In 17 women with infertility in this study, 10 cases had tubo-peritoneal adhesions and 6 cases had evidence of tubal occlusion that might definitely compromise their fertility. Early laparoscopy in PID also offers an opportunity to drain tubo-ovarian abscess or pyosalpinges and to perform a primary adhesiolysis.

Intrauterine contraceptive devices are considered to be a major risk factor for the development of pelvic inflammatory disease.[6,7] Chlamydia trachomatis is a major cause of pelvic inflammatory disease and its sequelae of ectopic pregnancy and tubal factor infertility. The effect of IUD use on chlamydial infections is not clear.[8] The association between a history of IUD use and a lower titre of chlamydial antibodies was observed in some studies of ectopic pregnancy.[9-11]

Previous studies reported that Chlamydia-infected IUD users were not at increased risk for PID when compared with uninfected IUD users and women not using a contraceptive method.[6-8] In the study reported here the differences between a group of IUD users and a control group of nonusers were not significant. Our findings support those of other investigators.

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