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Chlamydia Trachomatis Infection in Women with Ectopic Pregnancy

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ABSTRACT

Objective To evaluate the presence of chlamydial infection in the lower and upper genital tract of women with ectopic pregnancy .

Design Descriptive study.

Setting Department of Gynaecology, Guangzhou Maternal and Neonatal Hospital.

Subjects Forty women with ectopic pregnancy and thirty (age-matched) normally pregnant women served as controls were included.

Methods Using polymerase chain reaction (PCR) and enzyme immunoassay (EIA) to detect C. trachomatis in cervix and fallopian tube.

Results The rate of C. trachomatis in the women with ectopic pregnancy was significantly higher than in control group (37.5% vs 13.3%, $P < 0.05$). C. trachomatis in cervical samples were detected at a higher percentage than in tubal samples (37.5% vs 21.7%, $P > 0.05$).

Conclusion Our study suggested an association between chlamydial infection and ectopic pregnancy when compared with women with normal pregnancies.

Ectopic pregnancy (EP) is the most serious consequence of acute salpingitis. During the last decade, a rising incidence of EP has been reported in the developed world.[1,2] Although, not all the reasons for this increase in the incidence of EP are fully understood, the important risk factor is salpingitis. Chlamydia trchomatis is one of the most common etiologic agent causing salpingitis.[3,4] Several investigators have demonstrated that women with EP had significantly more chlamydial antibodies than normal controls.[5-7] The purpose of the present study was to identify

the presence of active chlamydia infection in the lower and upper genital tract of women who exprienced an exploratory laparotomy for ectopic pregnancy.

Materials and Methods

Between May 1995 and May 1996, forty women with ectopic pregnancy underwent laparotomy at the Department of Gynaecology, Maternal and Neonatal Hospital, Guangzhou, China. In 17 cases, the affected tube was removed, whereas in the remaining 23 patients, a more conservative surgical approach was chosen. The diagnosis of ectopic pregnancy was verified in all cases by histologic means of the involved tubal segment, which demonstrated chorionic villi in the tubal endothelium.

Thirty age-matched normally pregnant women were chosen as control subjects. All had intrauterine pregnancies and were confirmed by ultrasonography. All women (patients and controls), endocervical swab specimens, were obtained for chlamydial isolation.

From 34 women (46 specimens) with ectopic pregnancy, the fallopian tubes were swabbed on the mucosal surface of the fimbriae at the time of the surgical procedure.

Polymerase Chain Reaction (PCR) : Cervical and tubal samples were collected into sterile tubes containing 1.5 ml phosphate-buffered saline. The sample was agitated, transfered to a microcentrifuge tube and centrifuged at 10,000 rpm for 5 minutes. The pellet was then resuspended in 50 μl lysis buffer containing 0.45% NP 40, 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 45 sec, 72 °C 60 sec, 35 cycles, 72 °C 10 min. The amplified products were subjected to 1.5% agarose gel electrophoresis, and the DNA bands were viewed under ultraviolet light after staining with ethidium bromide. The presence of a 225 bp DNA band indicated the presence of C. trachomatis.

Enzyme Immunoassay : Endocervical and tubal 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 10-20 minutes with 0.6 ml Extraction Buffer containing 0.1% sodium azide, 5 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

The men age of the 40 patients with ectopic pregnancy was 31 years (range 20 to 38). 9 (22.5%) women had not been pregnant before, and 14 (35%) had never given birth. A history of salpingitis was reported by 14 patients (35%), and 5 women (12.5%) had experienced an episode of ectopic pregnancy. At least 1 year of infertility was reportd for 13 women (32.5%). At the time of conception, 8 women (20%) were using an IUD.

Endocervical Chlamydia trachomatis was detected in 15 of 40 patients (37.5%) with ectopic pregnancy by Polymerase Chain Reaction, compared with 4 of 30 in control (13.3%). This

difference was significant ($P < 0.05$). Chlamydia trachomatis was present in the cervix of 11 of 40 patients (27.5%) with ectopic pregnancy by EIA, and 3 of 30 controls (10%). In tubal samples, 10 of 46 (21.7%) were positive by PCR and 11 of 46 (23.9%) by EIA. Chlamydia trachomatis in the cervical samples were detected at a higher percentage (37.5%) than in tubal samples (21.7%). There was, however, no significant difference between the two sites ($P > 0.05$).

Section of fallopian tube tissue from the site of ectopic implantation were available from 32 cases. Eight (25%) had submucosal infiltration with lymphocytes and plasma cells.

Discussion

During the last decade, a number of seroepidemiologic studies have been presented in which the prevalence of chlamydial antibodies in women with ectopic pregnancy and various control groups has been compared. All the studies have shown significantly more antibodies in the group with ectopic pregnancy. In those studies, in which women with an intrauterine pregnancy were used as controls, some 47 to 82% of women with ectopic had chlamydial antibodies, compared with 13 to 58% of healthy pregnant women.[3,6-8]

The recent introduction of the PCR in our study demonstrated that Chlamydia trachomatis can be detected in the cervix and the fallopian tube. PCR is of interest because of a rapid response time and higher sensitivity in detecting Chlamydia.[9,10]

In the present study, 37.3% of the patients with ectopic pregnancy had positive results, compared with 13.3% of the controls, this difference was more pronounced for the patients in relation to its controls. By using the PCR technique and EIA, Chlamydial infection was identified in tubal specimens. Such 46 tubal specimens from 34 patients with ectopic pregnancy, 21.7% were positive by PCR and 23.9% by EIA. Therefore, there was a evidence of persistent Chlamydial infection in the tubes of these patients.

Osser and Persson[7] reported a history of salpingitis in 10% of the women, which is in accordance with other studies in which 6 to 25% of women with ectopic pregnancy had history of salpingitis. In our study, there was a higher percentage (35%) of the women. 25% were diagnosed in the tubes by pathology.

As had been suggested for tubal infertility, Chlamydia trachomatis may be the major aetiologic pathogen in cases of ectopic pregnancy. This study has suggested an association between chlamydia infection and ectopic pregnancy when compared with women with normal pregnancies. We found the evidence of chlamydial infection in the tubes of these patients by using PCR, but there was not significant difference between the cervix and the tube.

References

1. Ma Kinen JI. Increase of ectopic pregnancy in Finland-combination of time and cohort effects. Obstet Gynecol 1989 ; 73 : 21-4.
2. Ma Kinen JI, Erkkola RU, Laippala PJ. Causes of the increase in the incidence of ectopic pregnancy. Am J Obstet Gynecol 1989 ; 160 : 642-6.
3. Walters MD, Eddy CA, Gibbs RS, Schachter J, Holden AEC, Pauerstein CJ. Antibodies to chlamydia trachomatis and the risk of tubal pregnancy. Am J Obstet Gynecol 1988 ; 159 : 942-6.
4. Brunham RC, Binns McDowell J, Paraskevas M. Chlamydia trachomatis infection in women with ectopic pregnancy. Obstet Gynecol 1986 ; 67 : 722-6.
5. Svensson L, Mardh PA, Ahlgren M. Ectopic pregnancy and antibodies to chlamydia trachomatis. Fertil Steril 1985 ; 44 : 313-7.

6. Robertson JN, Hogston P, Ward ME. Gonococcal and chlamydial antibodies in ectopic and intrauterine pregnancy. Br J Obstet Gynaecol 1988 ; 95 : 711-6.
7. Osser S, Persson K. Chlamydial antibodies and deoxyribonucleic acid in patients with ectopic pregnancy. Fertil Steril 1992 ; 57 : 578-82.
8. Chow JM, Yonekura ML, Richwald GA, Greenland S, Sweet RS, Schachter J. The association between chlamydia trachomatis and ectopic pregnancy. JAMA 1990 ; 263 : 3164-7.
9. Witkin SS, Jeremias J, Toth M, Ledger WJ. Detection of chlamydia trachomatis by the polymerase chain reaction in the cervices of women with acute salpingitis. Am J Obstet Gynecol 1993 ; 168: 1438-42.
10. Wiesenfeld HC, Heine RP, Rideout A, Macio I, Dibiasi F, Sweet RL. The vaginal introitus : A novel site for chlamydia trachomatis testing in women. Am J Obstet Gynecol 1996 ; 174 : 1542-6.