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Prevalence of *Chlamydia trachomatis* Antigen and Antibody in Infertile Women in Ahwaz

Mohammad Javad Kajbaf* and Azita Gholamnezhad

Microbiology and Immunology Dept., Faculty of Medicine, Ahwaz Univ. of Medical sciences, Ahwaz, LR Iran

ABSTRACT

***Chlamydia trachomatis* is an obligate intracellular bacterium which causes a wide variety of human infections such as ocular, urogenital and respiratory infections. Genital infections of women, especially when repeated, give rise to many complications such as ectopic pregnancy, miscarriage and infertility. Since chlamydial infections are usually asymptomatic, they progress unnoticed and produce the sequela. The method of choice for the isolation of *C. trachomatis* is cell culture but other techniques like DIF and ELISA are more feasible, faster, less expensive and adequately specific and sensitive. In this study, prevalence of *C. trachomatis* antigen and antibody was assessed in a high risk group of infertile women by DIF and ELISA methods in Ahwaz, Southwest of Iran. The rates obtained were much less than those of many other populations, e. g. USA, Sweden, Australia, South Africa and Turkey. It was also concluded that presence of *C. trachomatis* antigen and antibody was associated with infertility in these women when compared with healthy pregnant control women.** *Iran. Biomed. J. 2: 45-48, 1998.*

Keywords: Chlamydia trachomatis, antigen, antibody, infertility

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INTRODUCTION

Chlamydia are obligate intracellular bacteria which cause a wide variety of infections in humans and animals. *Chlamydia trachomatis (C. trachomaits) is one* of three species of this group of bacteria that produces human infections in both men and women at all ages. These include trachoma, adult and neonatal inclusion conjunctivitis, neonatal pneumonia cervicitis, endometritis, salpingitis, nongonococcal urethritis, epididymitis, lymphogranuloma venereum (LGV) etc. [1]. Genital infections in women are more important and deserve full attention since they have some adverse consequences. Infertility and ectopic pregnancy are two major consequences. In 1980, ectopic pregnancy was reported in 1 in 107 pregnancies in Canada. These women frequently showed serologic evidence of *C. trachoma* infection and histologic evidence of salpingitis [2], Another study in a group of women with ectopic pregnancy who were undergoing laparotomy indicated that *C. trachomatis* might be a major cause of oviductal damage which predisposes to ectopic pregnancy [3]. Other consequence is effect on pregnancy. In a study of women with antepartum infection, the mean duration of gestation was significantly shorter than for non - infected controls. Moreover, stillbirth or neonatal death happened in 66% of infected women compared with 3.4% of control women [4]. A study on the prevalence of chlamydial infections in 1531 women from 10 clinics in New York State, excluding New York City, showed that overall chlamydial infection rates were 13.6% - 17.6% in 8 high risk family planning and sexually transmitted diseases (STD) clinics, and 5.7% in 2 low risk college and private clinics [5]. In this study, risk factors for chlamydial infection included: age older than 20 years, use of oral contraceptives and a history of having more than one sexual partner.

There are several methods for laboratory diagnosis of *C. Trachomatis* infections. Isolation of bacteria in cell culture is the method of choice and remains the most sensitive and specific one, although this method is expensive, laborious and needs special equipment. Some other techniques are direct fluorescent antibody (DFA) staining method, enzyme linked itnmunosorbent assay (ELISA) and gene probes. The Sensitivity and specificity of DFA and ELISA are around 90% when compared with cell culture. The advantage of these two methods is that they are fast, easy to perform and suitable for automation [6]. Detecting Chlamydial DNA in clinical specimens (Gene probe) is very sensitive, specific (both criteria>90%) and promising. This method which detects as little as one picogram of Chlamydia has been used for antigen detection in endocervical specimens [7]. Recently, polymerase chain reaction (PCR) has

*Corresponding Author

been successfully used to detect *C. trachomatis* in introital specimens as well as endocervical swabs in pregnant women in USA [8]. The detection rate for endocervical swab was 12.0% compared with introital specimens (11.7%) and it was shown that the sensitivity and specificity of introital specimen was nearly 100%. Clearly, introital specimens are much more easier to collect not only for adults but also for children. In present study, the first objective was to assess the prevalence of C. trachomatis antibody and antigen in a high risk group (infertile women) and control group (healthy pregnant women). The second objective was to find a correlation between infertility and presence of antigen and antibody.

MATERIALS AND METHODS

101 infertile women who had the history of infertility for more than one year and their respective husbands had normal sperm analysis test, were chosen as patient group. These patients had been referred to infertility clinics of 3 major Hospitals in Ahwaz city, Southwest of Iran. Control group consisted of 50 healthy pregnant women with no history of infertility. Two different samples were taken from every individual. Blood was taken for antibody detection test and endocervical smear was collected for antigen detection test.

Antibody detection. An ELISA system was used to detect specific IgG antibody to C. *trachomatis* in the serum. The ELISA kits were provided by the Labsystem (England). The procedure was carried out manually following the manufacturer's instructions. Briefly, serum was added to microplate wells containing fixed chlamydial antigen and incubated. After washing unbound antibody, anti-chlamydial IgG-alkaline phosphatase conjugate was added and incubated again another wash step was carried out and substrate, paranitrophenyl phosphate (PNPP), was added. After completion of incubation time, the reaction was stopped and the color intensity was read at 405 nm wavelength by an ELISA spectrophotometer.

Antigen detection. A sterile swab was used to collect endocervical epithelial cells which were spread on a clean glass slide in order to make a small film. The slide was then air dried and fixed by a drop of acetone which covered the film completely. DFA method was employed by using a Biomerieux kit (France) in which a fluorescein-conjugated monoclonal antibody against chlamydial antigen had been incorporated. Manufacturer's instructions were used to interpret the positive and negative results.

RESULTS

Chlamydial antibody. significant level of antibody was found in 18 (17.8%) infertile women, but only 3 (6%) women in control group were positive for this antibody. The difference between test and control group was statistically significant ($P<0.05$).

Chlamydial antigen. 8 (7.9%) women in infertile group had specific antigen in their endocervical smears, whereas 2 (4%) women in control group had the antigen. Again, the difference between the two groups was statistically significant ($P<0.05$). Results regarding antibody and antigen detection assays are shown in Table 1.

Clinical data. A questionnaire form was filled in for every woman in the test and control group. A lot of information was, therefore, gathered about age, length of marriage, primary and secondary infertility, hysterosalpingography and a history of hospitalization. These data were arranged as several tables and graphs and analyzed statistically. The Most important findings are summarized here. The age of the patients was ranging from 16 to 43 years and were divided into 6 groups. 60% of infertile women were in groups 19-25 and 26-30 years and the majority of antibody and antigen positive women were also in these 2 groups. According to the length of marriage, majority of infertile women had a length of 4 to 15 years, and these women formed the majority of antibody and antigen positive patients. 70% of infertile women had primary infertility versus 30% who had secondary infertility. Most antibody and antigen positive patients had secondary infertility.

All infertile women had hysterosalpingogram taken and several factors like tubal or uterus occlusion, salpingitis, etc., were looked for. Majority of patients had salpingitis who were also positive for antibody and antigen. The last clinical data which was considered was the question of hospitalization. Most patients (70%) did not have a history of hospitalization against 30% who had this experience.

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Table 1. Rate of *C. trachomatis* antibody and antigen in infertile (test) and healthy pregnant (control) women. Numbers inside the paranthesis are percentages.

C. trachomatis	Infertile women (101)	Healthy pregnant women (50)	*p* value
Ab positive	18 (17.8)	3 (6)	<0.05
Ab negative	83 (82.2)	47 (94)	
Ab positive	8 (7.9)	2 (4)	<0.05
Ab negative	93 (92.1)	48 (96)	

DISCUSSION

In this study, prevalance of chlamydial antigen and antibody was determined in a group of infertile women using two valuable methods. Other studies from around the world have also focused on the same subject. In Turkey, 39% of 185 infertile women had chlamydial antibody measured by indirect immunofluorescent antibody test (IFAT) and 13% had chlamydial antigen by enzyme immunoassay (EIA) method [9]. In South Africa, chlamydial antigen was measured in endocervical swabs by DIF method in 40 infertile and 40 healthy pregnant women. 35.9% of infertile and 3% of healthy pregnant women harbored the antigen, respectively [10]. In Taiwan, presence of chlamydial DNA was evaluated by DNA hybridization in endocervical swabs in infertile (186) and fertile (64) women. The antigen was found in 26.3% of infertile and 12.5% of fertile women [7]. In Sweden, 48% of 63 infertile and 13% of 55 healthy pregnant women had high antibody titer to *C. Trachomatis* [11]. In Melbourne, 45% of 110 infertile and 3% of 87 healthy pregnant women had *C. trachomatis* antibody [12]. In all these studies, prevalence of chlamydial antibody was ranging from 39% to 48% and chlamydial antigen from 13% to 35.9% in infertile women. The highest rate of antibody was seen in Sweden and that of antigen in South Africa.

In present study in Ahwaz, the prevalence of antibody was 17.8% and that of antigen was 7.9% in infertile women, respectively. These rates are much lower than those of other studies described here. As can be seen, rate of chlamydial antibody and antigen is completely varied in different geological populations. No definite explanation can be presented regarding these variations. It is, however, true that chlamydial infections are more frequently found in sexually active populations. These infections are now regarded, at least in industrialized societies, as the major cause of STD [13].

Chlamydial infections are usually asymptomatic and these patients are frequently the route of transmission. Over 50% of the infections in women are asymptomatic and hence progress undetected to silent pelvic inflammatory diseases (PID), infertility, ectopic pregnancy and infant morbidity [13]. Effective control strategies should, therefore, be employed as a priority at least for high risk populations. Although in the present study in Ahwaz the prevalence was not very high, compared with other populations, control measures are necessary since in industrialized societies it has been shown that screening, where the prevalence is 5% or more, is cost effective [13].

In the present study in Ahwaz, majority of infertile women who had salpingitis were also positive for antibody and antigen. Although no further attempt was made to show the presence of Chlamydia in fallopian tubes to ensure the association, other studies have proved this association. In Pennsylvania, a strong correlation was found between severity of tubal factor infertility and level of chlamydial antibody [14]. In another study in Seattle, a group of infertile women who had occluded fallopian tubes underwent surgery. In situ hybridization and immunoperoxidase staining was used to detect *C. trachomatis* DNA and antigen in biopsies, respectively. Majority of these women were positive for both tests indicating that C. *trachomatis* can persist in these tissues in an un-cultivable state [15]. Therefore, it is concluded that C. trachomatis infection might have played a role in infertility in Ahwaz.

The immune mechanism by which *C. trachomatis* infection affects fertility and pregnancy is not quite clear. Some investigators have measured antibody to *C. trachomatis* heat shock protein (CHSP 60) in women who underwent in vitro fertilization. In one study [16], antibody to CHSP 60 protein reduced the rate of pregnancy, whereas other study [17] showed that the same antibody dramatically increased the rate of pregnancy. The immunological process which leads to this controversial finding is not yet understood.

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