Chlamydia trachomatis antigen detection in female infertility

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***Background & objectives*: *Chlamydia trachomatis* is a well recognized sexually transmitted pathogen. Besides its potential to produce genital tract infection, *C. trachomatis* is increasingly being associated with long-term complications like infertility. The present study was undertaken to assess the role of *C. trachomatis* in female infertility .**

***Methods*: Women of primary and secondary infertility (n=150) and 20 healthy term pregnant women as control group were enrolled in the study. Detailed clinical history of each patient was recorded. Hysterosalpingography was performed in all patients. Endocervical swabs were collected for culture on cycloheximide treated McCoy cell line and for antigen detection by** Blocking assay antibody technique .

***Results*: *C. trachomatis* was detected in 48 (32%) of the 150 infertile women while 3 (15%) in control group was positive for *C. trachomatis* (*P*<0.02).** Among the total 48 (32%) infertile *Chlamydia* positive cases, *C. trachomatis* was detected by both cell culture and EIA, in 22 (45.8%), 14 cases (29.5%) were positive for *C. trachomatis* by cell culture alone and in 12 (25 %) only antigen could be detected.**Chlamydial positivity was seen in** 22(24.2%)**women with primary infertility and in** 26(44.1%)**with secondary infertility.**

***Conclusion*: A significantly high rate of *C. trachomatis* infection was found in infertile women and more so in asymptomatic females and in secondary infertility cases. Lack of symptoms make clinical diagnosis of chlamydial infection difficult. Screening of infertile women for *C. trachomatis* is therefore recommended so far early therapeutic interventions.**

Introduction

Over the past decade, the reported number of *Chlamydia trachomatis* infections has markedly increased, mostly due to increased screening activities. *Chlamydia trachomatis* infection, a prevalent sexually transmitted disease, is associated with complications like ectopic pregnancy, Fallopian tube block and adverse pregnancy outcome.[1], Chlamydial infection produces less severe symptoms than other sexually transmitted diseases[2]. These deceptively mild symptoms allow the infection to go unnoticed with minimal patient awareness until secondary or tertiary symptoms

develop. The sequelae of undetected and thus untreated infections like acute salpingitis and pelvic inflammatory disease lead not only to significant morbidity but far more importantly to infertility[3].
Chlamydia is a common sexually transmitted infection (STI) caused by the bacterium, *Chlamydia trachomatis*. Chlamydial infection is extremely common. *Chlamydia* is the most common sexually transmitted infection in the United States. The Center for Disease Control estimated that 2.8 million Americans are infected with *Chlamydia* each year[4].
Chlamydia can be transmitted during vaginal, anal, or oral sex. *Chlamydia* can also be passed from an infected mother to her baby during childbirth. Any sexually active person can be infected with *Chlamydia*. The greater the number of sex partners, the greater the risk of infection[5].
About 75% of infected women and about 50% of infected men have no symptoms of chlaymidal infection. If symptoms do occur, they usually appear within 1 to 3 weeks after exposure. Women who do have symptoms might have an abnormal vaginal discharge or a burning sensation when urinating. If the infection spreads from the cervix to the fallopian tubes some women still have no signs or symptoms; others have lower abdominal pain, low back pain, nausea, fever, pain during intercourse, or bleeding between menstrual periods. In women, untreated infection can spread into the fallopian tubes and cause the tubes to become blocked at the very ends (distal tubal obstruction). They can also develop scar tissue around the fallopian tubes that makes it more difficult for the tube to "pick up" the egg at the time of ovulation. The problems can lead to infertility and an increased risk for ectopic (tubal) pregnancy.

Distal tubal obstruction can be detected by performing a hysterosalpingogram. Pelvic adhesions, however, can only be detected by undergoing a laparoscopy to look inside of the abdominal cavity.Since laparoscopy is a much more invasive procedure, it is desirable to avoid it whenever possible. Infertility due to *C. trachomatis* represents a preventable type of infertility, if detected early. In majority of the women the infection with this organism is asymptomatic or with minimal symptoms[6]. Therefore, screening of women at risk is highly recommended[7].
Women at highest risk often have the least access to health care facilities. Therefore there is a need for a rapid, simple and accurate test to detect *C. trachomatis* infection, which can be performed outside the laboratory setting when the patient is still in the clinical setting.
Since 1996, the Royal College of Obstetricians has recommended that all `non-pregnant women under 35 years undergoing uterine instrumentation should be screened for *Chlamydia*, prior to the procedure or, failing that, should receive prophylactic antibiotics' . More recently, the Chief Medical Officer's Expert Advisory Group on *Chlamydia* . has called for action to reduce the prevalence and morbidity of chlamydial infection. They recommend that consideration be given to screening couples attending for fertility investigations and treatment[8]. Over the past 20 years, limited data regarding the prevalence of chlamydial infection in the subfertile population have emerged from prevalence studies within the UK and elsewhere. Comparing the studies, however, is difficult because of heterogeneity of sample size, criteria for case selection, diagnostic tests, and data collection. As a result, no specific data exist on which to base intervention strategies.

Detection of *C. trachomatis* infection by non-culture techniques became feasible with the recent development of immunologic reagents specific for *Chlamydia*. The direct

fluorescent antibody (DFA) staining and enzyme immunoassay (EIA) are two such tests[9].

*** The purpose of this study was to provide data on the prevalence of genital chlamydial infection in women attending for infertility investigation in MCH hospital , Buriadah , Alqassim region. We also studied the effect of age and diagnostic test on prevalence.**

Material & Methods

Infertile women of reproductive age attending Obstetrics and Gynaecology outpatient department of MCH, Buriadah , Alqassim region ,during May 2006 to October 2006 were included in the study. 20 healthy term pregnant women of similar age during the study period attending the antenatal clinic constituted the control group. Infertility was defined as inability to conceive for more than a year despite regular unprotected intercourse. Primary infertility was defined as those cases in whom conception had never occurred whereas the term secondary infertility was used to define those cases where there was inability to conceive after a previous successful conception. All infertile women and husbands having normal semen evaluation were enrolled in the study. Patients with history of antibiotic treatment in the previous two months were excluded from the study. Detailed history and clinical features were recorded and all relevant investigations were performed. Hysterosalpingography (HSG) was done in all cases. Study group comprised of 150 infertile women. They were further categorized on the basis of primary (91 cases) and secondary infertility (59 cases) and whether they presented with various symptoms like - vaginal discharge, Ectopic pregnancy , Bleeding per vaginum and Burning micturition. (73 patients) while were asymptomatic (77 cases).

Specimen collection

The specimen was collected as per the instructions of the kit manufacturer. To obtain a satisfactory specimen from the cervix the patient was examined in lithotomy position and the cervix was visualized using a bivalve speculum and wiped with sterile gauze held on sponge forceps so as to remove excess mucus/blood/pus etc. Endocervix was swabbed using a dacron tip swab provided with the kit. The swab was inserted approximately 1 cm into the cervical canal and rotated several times before withdrawing. The swab was removed without touching the vaginal surface and placed in 1 mL working strength transport medium in a heat resistant vial. Specimens were immediately transferred to the laboratory where they were stored at 2-8oC for no longer than seven days prior to testing.

Tissue culture technique, McCoy cell line used for the isolation of *C. trachomatis* was obtained from National Centre for Cell Science, Pune. The cell line was maintained in the laboratory according to standard technique. *C. trachomatis* was cultured on cycloheximide treated McCoy cell lines. One ml suspension of 1,00,000 McCoy cells/ml of growth medium was seeded in Leighton tubes containing cover slips. The tubes were incubated at 37oC in

a stationary position for 2-3 days for adequate growth to appear after which minimum essential medium (MEM) was aspirated from the vials and 0.1 ml from each 2SP specimen extract was inoculated into two tubes, one each for iodine and Giemsa

staining. Tubes were centrifuged at 2500-3000 *g* for 1 h after adding 1 ml of MEM containing 1μg/ml cycloheximide, the tubes were incubated at 37oC for 48-72 h. Inclusion bodies were detected by Giemsa and iodine staining .

Blocking assay antibody technique for detection of *Chlamydia* antigen , IDEIATM PCE *Chlamydia* and IDEIATM *Chlamydia* blocking reagent manufactured by DAKO Diagnostics Ltd. Cambridgeshire, UK were used. Former is an immunoassay using dual amplification technology for detection of *Chlamydia* antigen in endocervical swabs and the latter is a blocking antibody reagent, which is to be used in conjunction for verification of positive reactions.
The IDEIATM PCE *Chlamydia* blocking reagents consist of two reagents: a genus specific murine monoclonal antibody (blocking reagent) of different origin to that used in the IDEIATM PCE *Chlamydia* test and a murine monoclonal antibody with no anti-chlamydial activity (control reagent). The selective blocking test is performed on specimens found to be reactive in the IDEIATM PCE *Chlamydia* test. Each reactive specimen is retested using two wells in the IDEIATM PCE *Chlamydia* test. If *Chlamydia* LPS antigen is present in the specimen, the blocking antibody (but not the control antibody), will selectively bind to it and block the binding of Chlamydial LPS to the capture antibody on the coated well. On completion of the IDEIATM PCE *Chlamydia* test procedure, a significant reduction in the absorbance value obtained in wells containing the specimen or positive control and blocking reagent, relative to the wells containing the specimen or positive control and control reagent verifies the presence of *Chlamydia* LPS antigen. Adding 0.05 to the mean of the negative control values calculates the cut off value.

RESULTS

Overall, 150 women aged 17– 45 years were screened while attending the Obstetrics and Gynaecology outpatient department of MCH, Buriadah , Alqassim region ,during May 2006 to October 2006 were included in this study .
The mean age of the 150 women enrolled in this study was 23.9+ or - 5.54 yr while the mean age of the control group was 23.4+ or - 3.45.
Among the infertile cases 77 (51.33%) , there is ≤ 17 No. screened is 2 , from 21–25, No. screened is 15 , from 26–30 No. screened is 53 , from 31–35, No. screened is 46 ,and finally ≥ 36 No. screened is 34.
The healthy term pregnant control women were free of all signs and symptoms and their age distribution was similar to the study group.
Asymptomatic cases (n=77, 51.3%) slightl predominated in the study. Majority of the asymptomatic cases (n=52, 67.5%) had primary infertility while 25 (32.5%) had secondary infertility.
Among the (n=73, 48.6%) symptomatic cases there were 39 (53%) cases of primary infertility and 34(46.5%) cases of secondary infertility. The overall chlamydial positivity in the infertile women was found in 48 (32%) cases who were positive for one or both chlamydial markers while 3 healthy at term control women was found positive for *C. trachomatis* (P<0.02).
Among the total 48 (32%) infertile *Chlamydia* positive cases, *C. trachomatis* was detected by both cell culture and EIA, in 22 (45.8%), 14 cases (29.5%) were positive for *C. trachomatis* by cell culture alone and in 12 (25 %) only antigen could be detected.Taking culture as gold standard, Blocking assay antibody technique for

detection of *Chlamydia* antigen provides another alternative to culture. The reported sensitivity and specificity of these tests for genital infections (as compared to culture) have been 75-85% and 98-99% respectively in high risk populations. EIA offers the potential advantage over direct fluorescence assay (DFA) of objectivity and ease of mechanisation. It also allows batch processing, which is more conclusive in large scale screening. The best EIAs seem to have sensitivity similar to that of DFA in expert hands. However, non-culture tests are subject to false positive results. They should therefore be used with caution in low prevalence settings. Consequently, the interpretation of a positive test result must be handled with care and verification may be desirable.

In table 1 , show number of women tested by Blocking assay antibody technique for detection of *Chlamydia* antigen and Cell culture stratified by age for number screened, number positive.

In table 2 , show clinical profile of symptomatic infertile women (73cases) in relation to *Chlamydia* positivity.

In table 3 , show detection of *C. trachomatis* by Both cell culture and Blocking assay antibody technique and its relationships with Symptomatic women *and* Asymptomatic women infertility .

In table 4 , show detection of *C. trachomatis* by Both cell culture and Blocking assay antibody technique and its relationships with primary *and* secondary infertility .

Table I **Number of women tested by Blocking assay antibody technique for detection of *Chlamydia* antigen and Cell culture stratified by age for number screened, number positive.**

Age (years)	No. screened	No. positive (%)
≤ 17	2	1
21–25	15	4
26–30	53	13
31–35	46	14
≥ 36	34	16
Total	**150**	**48**

Table 2 **Clinical profile of symptomatic infertile women (73cases) in relation to *Chlamydia* positivity**

	No of cases(n=73)	Patients infected with *Chlamydia* (n=29)	Patients not infected with *Chlamydia* (n=44)
Vaginum discharge	25 (34.2%)	9 (36%)	16 (64%)
Chronic cervicitis	5 (6.8%)	2(40%)	3(60%)
Ectopic pregnancy	6 (8.2%)	1(16.6%)	5 (83.8%)
Burning micturition	7 (9.5%)	3(42.8%)	4(57.1%)
Scanty menses	9 (12.3%)	4(44.4%)	5 (55.5%)
Menorrhagia	13 (17.8%)	3(23.1%)	10(76.9%)
Bleeding per vaginum	8 (10.9%)	7 (87.5%)	1 (12.5%)

Table 3 **Detection of *C. trachomatis* by Both cell culture and Blocking assay antibody technique and its relationships with Symptomatic women *and* Asymptomatic women infertility .**

type	Total no. infected	Both cell culture and Blocking assay antibody technique	Cell culture	Detection by Blocking assay antibody technique
Symptomatic women (73=48.6%)	29(37.6%)	13(44.8%)	9(31%)	7(24.1%)
Asymptomatic women (77=51.3%)	19(26%)	9(47.4%)	5(26.3%)	5(26.3%)
total	**48**	**22**	**14**	**12**

Table 4 **Detection of *C. trachomatis* by Both cell culture and Blocking assay antibody technique and its relationships with primary *and* secondary infertility .**

type	Total no. infected	Both cell culture and Blocking assay antibody technique	Detection by Cell culture	Detection by Blocking assay antibody technique
primary infertility (91=60.6%)	22(24.2%)	12(54.5%)	5(22.7%)	5(22.7%)
secondary infertility (59=39.3%)	26(44.1%)	10(38.5%)	9(34.6%)	7(26.3%)
total	**48**	**22**	**14**	**12**

Discussion

Female genital tract is a suitable environment for growth of various pathogen and nonpathogen microorganisms. Some of the organisms such as *Chlamydia trachomatis* plays a role in a sexually transmitted disease[13].

Infertility is becoming an emerging health problem in many countries of the world[14]

C. trachomatis is an infectious agent in pregnant women. Depending upon the population studied and the method used for diagnosis the prevalence ranges from **2-37%** .Studies have proved a definite role of *C. trachomatis* infection in adverse pregnancy outcome. [8] If not treated on time their newborns run a 20-40% risk of developing chlamydial conjunctivitis[9] and a 10-20% risk of developing chlamydial pneumonia.[10], Studies from India have reported that 15% of young asymptomatic women are positive for this infection.[11] In this study, an effort was made to detect *C. trachomatis* antigen from the endocervical specimens of the pregnant women and to verify the positive results with the antibody blocking assay so as to achieve the most accurate results[16].

This study has been conducted on a selected population of women who themselves reported as infertile women. The duration of infertility in the Chlamydia positive cases in our study was approximately 3-5 yr which corresponds well with other reports.

A large number of the infected infertile women were asymptomatic Asymptomatic carriage of reported by us is a cause of concern. Although most infected women were asymptomatic , it has been reported earlier that at least half of infected people are carriers. The age of peak incidence is early twenties, which is sexually the most active age[15].
Genital infections caused by *Chlamydia* have been documented to be associated with abortions, whether spontaneous or medically terminated pregnancies. In another study was reported that **27.7%** positivity. It is this group that forms a high risk population and requires screening to avoid post abortal complications like ectopic pregnancy and secondary infertility. It was reported that a very high incidence of 38.5% in other study, which was based on detection of IgM and IgG antibodies. Women experiencing recurrent spontaneous abortions have high titres of anti chlamydial IgG but negative endocervical cultures for *C. trachomatis.*
The incidence of *C. trachomatis* infection was more common in women with secondary infertility.
This increased susceptibility could be due to their longer period of active sexual life thus enhancing their exposure to chlamydial infection. Secondary infertility associated with higher rates of chlamydial infection have been reported earlier by others[13].Bleeding per vaginum (on touch) and vaginal discharge were found to be more common clinical presentations in symptomatic chlamydia positive cases[16].

In other study, it was demonstrated that when the attendees of the gynecological department were divided into those with self-reported genital symptoms and those without such symptoms, the prevalence of genital *C. trachomatis* infection was **13% *vs.* 5%, respectively.**
In the present study the overall prevalence of genital *C. trachomatis* infection was found to be 48 cases from 150 (32%).
Owing to the asymptomatic nature of lower genital tract infection with *C.trachomatis*, most infected women can only be identified by screening. It is well known that those who undergo uterine instrumentation are at risk of upper tract dissemination of endocervical chlamydial infection[17]. Those undergoing infertility investigation and treatment may be at risk through hysterosalpingography, laparoscopy and dye hysteroscopy, intrauterine insemination, and/or embryo transfer. It is therefore not surprising that the Chief Medical Officer's Expert Advisory Group advised that consideration be given to screening couples with fertility problems .
Prevalence of *C. trachomatis* varies with the population under study and the sensitivity of the laboratory method used. Our study suggests that all infertile women should be screened for *C. trachomatis*. The index of suspicion should be higher in asymptomatic women in whom our study revealed a larger chlamydial positivity. In the absence of requisite infrastructure and skills for culture and for direct fluorescent assay, Blocking assay antibody technique for detection of *Chlamydia* antigen can play a significant role in screening for *C. trachomatis* in infertile women. Screening of infertile women for *C. trachomatis* is recommended in the first year of infertility itself so that early therapeutic intervention can be instituted to allow women to conceive naturally.

Blocking assay antibody technique for detection of *Chlamydia* antigen provides another alternative to culture. The reported sensitivity and specificity of these tests for genital infections (as compared to culture) have been 75-85% and 98-99% respectively in high risk populations. EIA offers the potential advantage over direct

fluorescence assay (DFA) of objectivity and ease of mechanisation. It also allows batch processing, which is more conclusive in large scale screening. The best EIAs seem to have sensitivity similar to that of DFA in expert hands. However, non-culture tests are subject to false positive results. They should therefore be used with caution in low prevalence settings. Consequently, the interpretation of a positive test result must be handled with care and verification may be desirable.

***Conclusion*: A significantly high rate of *C. trachomatis* infection was found in infertile women and more so in asymptomatic females and in secondary infertility cases. Lack of symptoms make clinical diagnosis of chlamydial infection difficult. Screening of infertile women for *C. trachomatis* is therefore recommended so far early therapeutic interventions.**

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