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Study of Chlamydia Trachomatis in Infertile Women

*Dr. Durgesh Gopalrao Deshmukh ** Dr Jyoti K Bajaj
Dr. Damle Ajit S * Dr. Jayanti Mania-Pramanik
*****Dr. Sonali Deshpande ****** Shilpa C. Kerkar

* Assistant professor Department of Microbiology, Shri Vasantrao Naik Government Medical College, Yavatmal, Maharashtra, India 445001

** Associate professor Microbiology, Government Medical College, Aurangabad

*** Professor and head of the Department Microbiology, Government Medical College, Aurangabad

**** National Institute for Research in Reproductive Health (Indian Council of Medical Research),J.M. Street, Parel, Mumbai-400030, India

***** Associate professor Obstetrics and Gynecology, Government Medical College, Aurangabad, Maharashtra, India

***** National Institute for Research in Reproductive Health (Indian Council of Medical Research),J.M. Street, Parel, Mumbai-400030, India

ABSTRACT

Infertility is a worldwide problem and tubal factor infertility important cause of infertility. One of most common cause of tubal factor infertility is pelvic inflammatory disease (PID). Chlamydia trachomatis (CT) is important cause of pelvic inflammatory disease, patient present to physician when complication of disease already occurs because most of the infections are asymptomatic. So we undertook this study to determine association of previous C.trachomatis infection and tubal factor infertility with the help of serology and PCR and also to determine the association of C.trachomatis infection and secondary infertility with previous history of spontaneous Abortion or ectopic pregnancy.

Method -: The study group was recruited from women of reproductive age less than 35 years with primary and secondary infertility that came to the obstetrics and gynecology outpatient department. Hysterosalpingography (HSG) used to asses fallopian tube .CT-IgG done to fined previous CT infection and CT-PCR done to see current infection. Also CT-IgG compare with Hysterosalpingography to predict fallopian tubal blockage.

Result-: Total 116 patients involve in the study 60 patients of primary infertility and 56 patients of secondary infertility. Total 32 patients show tubal obstruction on hysteronsalphyngrophy. Significant association was found between CT IgG positivity and secondary infertility. Significant association was found between CT IgG, CT PCR positivity and tubal factor infertility and also significant association find between CT-IgG positivity and secondary infertility patients with previous history of abortion and ectopic pregnancy. CT-IgG can predict tubal blockage in infertile patient.

Conclusion –: Chlamydia trachomatis infection is important cause of PID and infertility. Its early detection prevents its complication like infertility, abortion and ectopic pregnancy. CT-IgG can use for predicting tubal factor infertility so unnecessary invasive procedure can be avoided in these patients

Keywords : Chlamydia trachomatis , pelvic inflammatory disease, Infertility

Introduction

Infertility is a worldwide problem. 15-20% married couples are childless though they want children. The tubal factor is the most frequent cause of female infertility. Tubal disease is responsible for 25–35% of female infertility.[1] One of the most preventable causes of infertility is sexually transmitted diseases. *Chlamydia trachomatis* belongs to the most common sexually transmitted bacterial organisms, worldwide. The bulk of infections remains undetected and untreated because most infected people are asymptomatic and do not seek medical attendance. If untreated, *chlamydiae* may reach the upper genital tract of affected women and cause pelvic inflammatory disease (PID).[2] Pelvic inflammatory disease (PID) is the most common cause of tubal disease, representing greater than 50% of cases [3,4] After one episode of PID, the rate of infertility has been estimated at 11%, which increases to 23% and 54% after two and three episodes, respectively.[5] Similarly, multiple studies have shown associations between previous *chlamydial* infection, both symptomatic and asymptomatic, and abortions , ectopic pregnancy.[6]In some industrialized countries screening programs have been established to reduce the rate of PID and to prevent development of reproductive sequelae.[7,8,9]

So we undertook this study to determine association of previous *C.trachomatis* infection and tubal factor infertility with the help of serology and PCR and also to determine the association of *C.trachomatis* infection and secondary infertility with previous history of spontaneous Abortion or ectopic pregnancy.

MATERIALS AND METHODS

Study was conducted over period of 1year from Jun 2009 to Jun 2010.

The study group was recruited from women of reproductive age less than 35 years with primary and secondary infertility that came to the obstetrics and gynecology outpatient department of Government Medical College, Aurangabad, India. They had no genital anomaly, hormonal imbalance , galactorrhea, hirsutisum, diabetes, hypertension, thyrotoxicosis, spouse sperm count within normal limits and no contraceptive use. Detailed history and clinical features were recorded, and all relevant investigations were performed.

Hysterosalpingography (HSG) was done in all cases subsequent to enrollment. Tubal infertility was said to be present if bilateral tubal occlusion with or without hydrosalpinx was seen on HSG. They were further categorized on the basis of whether they presented with symptoms suggestive of urogenital tract infection or had no symptoms. Secondary infertile patients were further divided into whether patient had previous history of spontaneous Abortion or ectopic pregnancy or not.

Specimen collection- After cleaning the cervix, two cervical swab Specimen were taken in HDPE tube from HiMedia Laboratories. Swabs were immediately transported to microbiology department in CT transport medium from Sacace biotechnology (18 San Carlo str.,81100 Caseta,Italy) for Chlamydia trachomatis specific polymerase chain reaction (PCR). Blood Specimen was collected in a plain tube and sent to microbiology department for detection of *Chlamydia trachomatis* specific antibody by ELISA.

Laboratory diagnosis of *C trachomatis*.
DNA was extracted from the swab specimen in the molecular biology section of microbiology department using DNA-Sorb-A extraction kit from Sacace Biotechnologies (18 San Carlo str.,81100 Caseta,Italy) as per the manufactures instruction.

C. trachomatis was amplified using Seeplex HPV/STD4 ACE Screening Multiplex PCR kit (Taewon Bldg.65-5, Bangyi-Dong, Songpal-Gu, Seoul 138-050, Korea) as per the manufacture instruction. It amplifies *Chlamydial* 711bp plasmid DNA with amplification program as follows; initial denaturation 94 ºC for 15 min, followed by forty cycles of denaturation 94 ºC for 30 sec, Annealing 60 ºC for 1min 30 sec, Elongation 72 ºC for 1min 30 sec and final step of elongation 72 ºC for 10 min. Electrophoresis of amplified product was run on 2% Agarose gel , observed under UV transilluminator and the results were documented.(figure 2)

Duplicate cervical swabs were sent to National Institute for Research in Reproductive Health, Indian Council of Medical Research, Mumbai, India as part of quality control program, where. DNA was extracted from the swab specimen using a rapid non-enzymatic method.[10] Polymerase Chain Reaction (PCR) amplification of in-house β-globin gene was carried out to identify sampling errors and to monitor the absence of PCR inhibitors in the extracted genomic DNA.PCR was performed on extracted DNA using a primer pair selected from the conserved region of the Major Outer Membrane Protein (MOMP) gene of *C.* trachomatis [11] following a standardized PCR protocol.[12] This primer pair amplifies an 180bp DNA fragment, which is common to all serotypes of *C. trachomatis*. Electrophoresis of the amplified products was carried out on 2% agarose gel containing Ethidium bromide and observed under an UV transilluminator and the results were documented. Standard protocol for southern blotting was then carried out for transfer of PCR products to nylon membrane, which was further processed for hybridization using a *C. trachomatis* specific probe. PCR dig-labeling reagent (Roche diagnostics G_{mb}H, Mannheim, Germany) was used to synthesize probe. Immunodetection was carried out using dig-luminescence detection reagents (Roche diagnostics G_{mb}H, Mannheim, Germany). (figure 3) All our PCR results co-related with the NIRRH PCR results.

Detection of *C. trachomatis* IgG Antibodies
Commercially available enzyme-linked immunosorbent assay (ELISA) kit was used to detect *C.trachomatis* specific IgG antibody. (NovaTec Immunodiagnostica, G_{MB}H). In brief, Microtitre wells pre coated with *C. trachomatis* antigens were incubated with serum specimen at a 1:100 dilution so that any corresponding antibodies if present in the serum would bind to antigen to form complexes. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) labeled anti-human IgG conjugate was added which binds to captured Chlamydia specific antibodies. The immune complex formed by the bound conjugate was visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue colored reaction product. After terminating the reaction using a stop solution (sulphuric acid, 0.2mol/l), the absorbance of the end product, which is yellow in color, was read at 450 nm using an ELISA plate reader (μ Quant, Bio-Tek Instruments, Inc.). The intensity of this product is directly proportional to the amount of Chlamydia-specific IgG antibodies in the specimen. The specimens with O.D. higher than the cut off value (0.250-0.900) were considered positive for Chlamydia specific antibodies and used as an indicator of past Chlamydia infection.

Statistic analysis – Statistical analysis was performed using Epi info software version 3.2 Data was compared with the chi square test. A P value of <0.05 was considered statistically significant.

RESULTS
Out of 252 infertile women total 116 fulfill the study criteria they were enrolled in the study. Among these infertile cases, 54 (44.82%) were in the age group of 26-30 years,50 (41.37%) were in 20-25 year age group and 12 (13.79%) were in 31-35 year age group. 68 (58.62%) women belonged to urban area and 48(41.37%) belonged to rural area. 60 (51.72%) women had primary infertility and 56(48.27%) women had secondary infertility. 24(20.68%) women had history of previous abdominal pain and viginal discharge. Out of 56 secondary infertile women 28(50%) had Spontaneous Abortion and ectopic pregnancy, out of these 12(42.86%) women had history of 1st trimester abortion, 4(14.28%) women had history of 2nd trimester abortion,4(14.28%) women had history of recurrent abortion,6(21.42%)women had history of ectopic pregnancy, 2(7.14%) women had history of recurrent abortion with ectopic pregnancy. (Table1)

Table1 Patients with Spontaneous Abortion or ectopic pregnancy (n-28)

History	No. patients
1st trimester abortion	12 (42.86%)
2nd trimester abortion	4 (14.28%)
Recurrent abortion	4 (14.28%)
Ectopic pregnancy	6 (21.42%)
Recurrent abortion with ectopic pregnancy	2 (7.14%)

32 (27.58%) women showed fallopian tube blockage on hysterosalpingography, out of which 24(75%) women had secondary infertility and 8(25%) women had primary infertility. Total 40 patients come positive for C.trachomatis IgG (CT-IgG) of which 32 (57.14%) patients of secondary infertility and 8 (13.33%) patients of primary infertility. A statistical significant association was observed when *C.trachomatis* IgG positive patients were compared with primary vs secondary infertility (Chi-square -10.44 P –Value- < 0.05) (Table 2) .Out of 32 women with tubal factor infertility (TIF), 24 (75%) were positive for *C.trachomatis* IgG , of which 20 (83.33%) women had secondary infertility and 4(16.6%) women had primary infertility. A higher percentage of *C.trachomatis* IgG positivity was seen in patients with TIF which was statistically significant when compared to patients without TFI. (Chi-square-7.16 , P -Value -< 0.05). (Table 2). It was seen that out of 28 women of secondary infertility with spontaneous Abortion or ectopic pregnancy,24 (85.71%) were positive for CT IgG antibody. All 6 women with previous history of ectopic pregnancy were CT-IgG positive. (ref both table 1 and table 2) .A statistical significant association was observed when *C.trachomatis* IgG posi-

tive secondary infertile patients were compared with previous history of spontaneous Abortion or ectopic pregnancy vs without history. (Chi-square-14.36, P –Value-<0.01) (Table 2)

Total 16 infertile women come positive for *C.trachomatis* PCR of these 8 patients of secondary infertility and 4 patients of primary infertility positive for both *C.trachomatis* PCR and *C.trachomatis* IgG. Out of 32 women with Tubal factor infertility 12(37.5%) were positive by PCR; 8(66.66%) secondary infertility 4(33.33%) primary infertility. Difference between positive for CT-PCR in patient with tubal factor infertility and patient with absent tubal factor infertility was statistically significant. (Chi-square-4.82,P -Value -< 0.05). (Table 2)

Table 2 – results of bivariant analysis to asses the association between CT-IgG, CT-PCR positivity and various characteristics of women with infertility

Characteristics	Total No. of patient	CT-IgG positivity	P value	CT-PCR positivity	P value
Type of infertility (n-116) Primary infertility Secondary infertility	 60 56	 8(13.33%) 32(57.14%)	 < 0.05	 8(13.33%) 8(14.28%)	 >0.05
Tubal factor infertility (n-116) Present Absent	 32 84	 24 (75%) 16(19.04%)	 < 0.05	 12 (37.5%) 4(4.76%)	 <0.05
Spontaneous Abortion/ectopic (n-56) Present Absent	 28 28	 24(85.71%) 8 (28.57%)	 < 0.01	 4(14.28%) 4(14.28)	 >0.05

Considering HSG as marker of tubal blockage, sensitivity of Chlamydial IgG antibodies as a diagnostic marker for tubal factor infertility was 75%, and specificity 80.95%. (Table 3)

(Table 3)-Comparison of Chlamydia IgG antibodies and HSG as markers of tubal blockages.

CT IgG	HSG-positive cases,	HSG-negative cases,	Total
CT IgG positive cases	24 (75%)	16 (19.04%)	40
CT IgG Negative cases	8(25%)	68(80.95%)	76
Total	32	84	116

Sensitivity =75% specificity= 80.95%

Positive predictive value = 60%, Negative predictive value=89.47%

DISCUSSION

Upper reproductive tract infection with *C. trachomatis* is recognized to be a major cause of tubal-factor infertility and ectopic pregnancy among women. It is Important to elucidate the cause of tubal dysfunction in view of the problem.[2] In our study *C trachomatis* IgG and PCR positivity was significantly present in women with secondary infertility (p< 0.05). Women with secondary infertility also had more prevalence of tubal factor infertility (p<0.05). Findings of present study were similar to Malik et al[13] from India where they reported that 55% of secondary infertile women were positive for *Chlamydia trachomatis IgG* antibodies. Several studies have documented association between infertility and tubal pathology and the presence of circulating antibody to *C.trachomatis.* [14,15,16,17]

We found *Chlamydia trachomatis* IgG more frequently in patients with abortion and ectopic pregnancy. All the three patients with ectopic pregnancy were *C.trachomatis*-IgG positive. Several studies have documented association between spontaneous abortion and ectopic pregnancy with *C.trachomatis* infection. Malik et al[18] found *C.trachomatis* IgG antibodies in 45%. Sharma et al[19] 50%. Quinn PA et al[20] 57.6% and Shivananda P R et al[21]55.5% of bad obstetrics history patient respectively.

It has been seen that *Chlamydia trachomatis* courses inflammation of fallopian tube and uterine cavity that results in increase in concentration of cytokines in local region. Despite the unresolved nature of the disease etiology, persistent model of Chlamydial infection has been studied to provide insight into the nature of chronic disease.[22, 23].

Persistence is defined as a long-term association between Chlamydia and their host cell in which these organisms remain in a viable but culture negative state[22, 23] Chlamydial persistence is thought to be due in part to a failure to undergo secondary differentiation from RB to EB[22].

When we correlate CT-IgG as predictor of infertility with HCG sensitivity and specificity of CT-IgG come out to be 85% and 75% respectively. Hence CT-IgG detection can be used as routine procedure for infertility work up. As invasive procedures like Hystero Salpingography (HSG) and laparoscopy require to diagnose tubal-factor infertility, multiple studies evaluate serological testing of C*. trachomatis* to predict tubal factor infertility.

Malik et al[13] from India reported sensitivity of Chlamydial IgG antibodies as a diagnostic marker for tubal factor infertility which was 72.7% and specificity was 77.7%

Veenemans et.al[24] compared the results of *C.trachomatis* antibody testing and HSG with respect to their predictive value of tubal factor infertility. He found results were comparable.

Dabekausen et.al[25] reported that *C.trachomatis* antibody testing is more accurate than HSG in predicting tubal factor infertility.

Keltz et al[26] reported sensitivity and the specificity of *Chlamydia* antibody testing to be 74% and 93% respectively.

Laparoscopy with tubal patency testing remains the most accurate method of diagnosing tuboperitoneal pathology at the moment .HSG is used for screening purposes. Patient tends to experience the procedure as painful and an annoying test. Furthermore, HSG has an infection risk of 1-3%. The Chlamydial IgG antibodies on the other hand, is a simple blood test and causes little inconvenience for the patient.[24]

Malik et al[13.] suggested that in a resource-poor country such as India ELISA for Chlamydia IgG antibodies can easily be substitute for HSG as a screening test for detection of tubal occlusion and it should be maintained in infertility work- up.

Keltz et al[26] showed that high positive predictive value (94.8%) of *Chlamydia* serology makes it a good screening test before laparoscopy. However, its low negative predictive value of 69.8% necessitates the continued use of HSG to find tubal damage from causes other than *Chlamydia*. They also showed that HSG and *Chlamydia* serology when combined, their sensitivity and specificity for finding tubal pathology was increased to about 80%. *Chlamydia* serology is an inexpensive, noninvasive test for screening of tubal factor infertility that may help directly in the management of infertile patients. Except to diagnose endometriosis in patients with menstrual dysfunction and lower abdominal pain, laparoscopy is unlikely to add to the diagnostic work-up for infertility. Likewise, when either the *Chlamydia* serology or HSG is abnormal, laparoscopic tubal assessment is indicated.

CONCLUSION

The present study finding show that specific antichlamydial antibodies are more frequently detected in infertile patient with Chlamydia infection as compared to direct method like PCR ,In present study, we could demonstrate that *C. trachomatis* infection must be also considered as an important risk factor for infertility, ectopic pregnancy and abortionc history patients. Present study confirms the previous finding that tubal abnormalities are important cause of infertility in woman.[2, 14 to 17] We also found that women with infertility or ectopic pregnancy associated with tubal diseases had much higher prevalence CT IgG antibody and its detection must be incorporated in infertility work up.

Finally we should have screen strategies least in high risk group to detect Chlamydia infection early which will prevents its later development of reproductive sequelae.

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