Chlamydia trachomatis infection in Infertile Women in North India: Diagnostic efficacy of an In-house Real-Time PCR assay

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Introduction

- Infertility is becoming an emerging health problem in many countries
- *Chlamydia trachomatis* not only jeopardizes fertility but also poses risk for infertility treatment and resulting pregnancies

Aims and Objectives

- To evaluate the diagnostic utility of an In-house Real-Time PCR assay for detection of *C. trachomatis*
- To determine the prevalence of *C. trachomatis* in Indian women attending an infertility clinic and identify factors associated with infection

Materials and Methods

I. Study population: Two-hundred sixty four infertile women aged 22-40 years
Exclusion criteria: Women with
   a) Male factor infertility
   b) Infertilty due to *M. tuberculosis* and *N. gonorrhoeae*
   c) Polycystic Ovary Syndrome (PCOS)
   d) Fibroid

II. Samples: Endocervical swabs

III. Microbiological methods:
Antigen detection for *C. trachomatis*:
   a) DFA Testing (Microtrak, USA)
   b) ELISA (Microtrak ii, USA)

PCR Assays:
   a) Cryptic plasmid and *omp*1 gene PCR
   b) IHRT-PCR (targetting 71 bp DNA segment)
   c) Cobas-Taqman CT Test v 2.0 (Roche Diagnostics, USA)

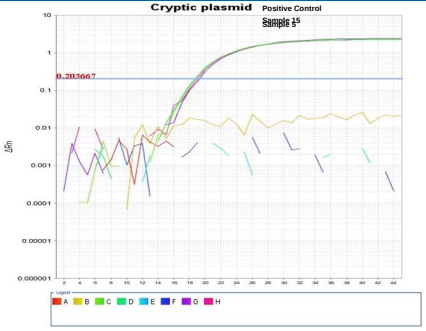

Fig II: Amplification Plot of in-house real-time PCR(ΔRn vs. Cycle)
A and B: Negative control
C and D: Positive clinical sample
D and E: Negative clinical sample

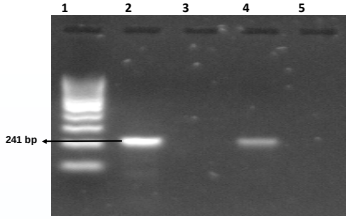

Fig 2 : PCR for *C. trachomatis* (Cryptic plasmid)
Lane 1 : 100 bp DNA ladder
Lane 2 : Positive control, Serovar D (ATCC VR 885)
Lane 3 : Negative control
Lane 4 : Clinical sample - positive
Lane 5 : Clinical sample - negative

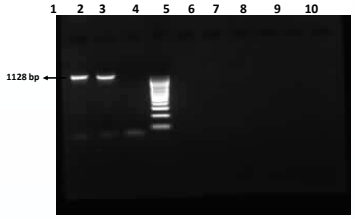

Fig 3: PCR for *omp*1 gene
Lane 1: Positive control
Lane 2: Clinical sample - positive
Lane 3: Negative control
Lane 4: DNA Molecular size marker (100bp-1kb)

Results

- There was no significant correlation between rates of infection and age group (Table 2)
- Detection of *C. trachomatis* antigen by DFA and ELISA was 9.0% and 6.5% respectively
- *C. trachomatis* was detected in 13.5% of infertile women by Real-Time PCR (Fig.1) as compared to 11.5% by both Cryptic plasmid (Fig.2) and *omp* 1 gene PCR (Fig.3)
- The sensitivity of ELISA and DFA was 12.5% and 18.8% respectively taking Cryptic plasmid PCR and *omp* 1 gene PCR as gold standard method (Table 1)
- The sensitivity and specificity of the IHRT-PCR was 100% considering COBAS Taqman as the gold standard (Table 1)

Table 1: Comparison of IHRT-PCR, cryptic plasmid and *omp* 1 gene PCR, DFA and EIA assays for *C. trachomatis* with COBAS Taqman CT test, v 2.0 (n=57)

Assay	Result	Cobas Taqman CT Test, v2.0 Results		Sensitivity % (95% CI)	Specificity % (95% CI)	Positive predictive value % (95% CI)	Negative predictive value % (95% CI)	Diagnostic accuracyn % (95% CI)
		Positive	Negative					
In-house Real-time PCR	Positive	27	0	100 (87.5-100)	100 (88.7-100)	100 (87.5-88.6)	100 (88.7-100)	100 (93.6-100)
	Negative	0	30					
Cryptic plasmid and/ or omp1 gene PCR	Positive	23	0	85.2 (67.5-94.1)	100 (88.6-100)	100 (85.7-100)	88.2 (73.4-95.3)	93 (88.3-97.2)
	Negative	4	30					
DFA	Positive	18	0	66.7 (47.8-87.3)	100 (88.6-100)	100 (82.4-100)	76.9 (61.7-87.4)	84.2 (72.6-91.5)
	Negative	9	30					
EIA	Positive	13	0	48.2 (30.7-66)	100 (88.7 – 100)	100 (77.2-100)	68.2 (53.4-80)	75.4 (62.9-84.8)
	Negative	14	30					

Table 2: Demographic and clinical characteristics of infertile women with and without *C. trachomatis* infection (n=200)

Characteristics	*C. trachomatis* Positive group, no (%) [n=27]	*C. trachomatis* Negative group, no (%) [n=173]	*P*-value
Demographic factors			
Age in years			
- < 25	5 (18.5 %)	11 (6.4%)	0.13
- 25-29	12 (44.4%)	70 (40.5%)	
- 30-34	7 (25.9%)	59 (34.0%)	
- ≥35	3 (11.2%)	33 (19.1%)	
Clinical characteristics			
Vaginal discharge			
-Yes	4 (14.8%)	16 (10.9%)	0.37
-No	23 (85.2%)	157 (89.1%)	
Abdominal pain			
-Yes	2 (7.4%)	19 (89.9%)	0.57
-No	25 (92.6%)	154 (86.3%)	
History of Abortion			
-Yes	11 (40.7%)	67 (38.7%)	0.85
-No	16 (59.3%)	106 (61.3%)	
History of ectopic pregnancy			
-Yes	4 (14.8%)	22 (12.7%)	0.76
-No	23 (85.2%)	151 (87.3%)	
Type of infertility			
- Primary	10 (30%)	81(46.8%)	0.34
- Secondary	17(70%)	92 (53.2%)	
Causes of infertility			
- Tubal	23 (85.2%)	133 (76.9%)	0.76
-Ovarian	2 (7.4%)	19(10.9%)	
-Endometriosis	0 (0%)	4 (2.4%)	
-Unexplained	2 (7.4%)	17 (9.8%)	

Discussion

- *C. trachomatis* is a major concern for reproductive health of women
- The high prevalence of infection in Indian women with infertility emphasizes the importance of timely diagnosis to avoid infection sequelae. Screening women for *C. trachomatis* should be a part of routine investigations for infertility
- Nucleic-acid amplification tests superseded the established Antigen-detection methods routinely used in Indian hospitals for detection of *C. trachomatis*
- The IHRT-PCR used in our study exhibited an excellent sensitivity and specificity similar to commercial NAAT based assays
- Given its low-cost , IHRT-PCR assay has the potential for screening of infertile women for *C. trachomatis* infection in resource limited settings

Acknowledgement

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