Full Length Research Paper

Value of direct antigen detection methods in diagnosing women with suspected genital *Chlamydia trachomatis* infection

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Establishing a method that would allow the quick and cost- effective diagnosis of *Chlamydia trachomatis* (*C. trachomatis*) is of highest interest. We aimed to evaluate the diagnostic efficacy of direct antigen detection methods [direct fluorescent antigen detection (DFA), Enzyme-linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR)] in comparison to culture method, to establish the most reliable and easy technique for diagnosing of *C. trachomatis* in females with suspected infection. Seventy patients were selected from females attending Outpatient Gynecology Clinic, Mansoura University Hospital, who were consulting for symptoms suggestive of genital infection. Two cervical swabs were taken from each patient and examined for *C. trachomatis* by direct detection methods. McCoy cell culture detected by immunofluorescence was positive in 16 cases (gold standard). Direct fluorescent antigen detection (DFA), ELISA and PCR were compared to McCoy cell culture in terms of sensitivity, specificity, accuracy, positive and negative predictive values. Sensitivity of DFA was lower than its specificity. Antigen detection by ELISA was positive in 28 (40%) cases. NPV (83.33%) and PPV (32.14%). Sensitivity of PCR compared to culture was 81.25% and specificity was 90.74%. In conclusion, McCoy cell culture assay is the most reliable test but tedious. Combination of PCR and DFA tests could optimize diagnosis of female genital *C. trachomatis* infection. Reevaluation of ELISA depending upon multiple tests as gold standard may increase its sensitivity and specificity.

Key words: *Chalmydia trachomatis,* direct antigen detection, polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA).

INTRODUCTION

Chlamydia Trachomatis is the most prevalent sexually transmitted pathogen worldwide. It is common among sexually active young women (Bauwens et al., 1993). In women, 75% of Chlamydia infections are asymptomatic and can lead to pelvic inflammatory disease (PID), infertility and ectopic pregnancy. Infants exposed to infection at birth also have a high risk to develop conjunctivitis and pneumonia. The asymptomatic nature of the disease means that treatment is often delayed, leading to an increased risk of complications and transmission to the other partner (Bébéar and Barbeyrac, 2009; Black, 1997).

Early diagnosis is mandatory to avoid serious complications especially with the development of effective treatment. Confirmation of Chlamydia infection usually depends on taking an appropriate specimen and a suitable laboratory-based diagnostic test (Boyadzhyan

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et al., 2004). As *C. trachomatis* is an obligate intracellular pathogen, cell culture remains the reference method and it has 100% specificity but it is not recommended for routine use because of its technical complexity, the long turn-around time and it is unsuitable in developing countries. Therefore many commercial non-culture-based assays are now available for diagnosis (Carder et al., 2006).

During 2007, approximately 1.1 million cases of Chlamydia were reported to CDC; more than half of these were in females aged 15 to 25 years (Center for Disease Control and Prevention (CDC), 2009).

There is no previous data about prevalence of *C. trachomatis* infection in Egyptian females. In this work we tried to evaluate the diagnostic efficacy of direct antigen detection methods, including DFA, ELISA and PCR in terms of sensitivity, specificity, accuracy, positive and negative predictive values compared to culture method to establish the most reliable and easy technique for diagnosis of *C. trachomatis* in Egyptian females with suspected infection.

MATERIALS AND METHODS

The present study was carried out during the period from March 2007 to January 2009. Seventy patients were selected from females attending Outpatient Gynecology Clinic, Mansoura University Hospital, who were consulting for symptoms suggestive of genital infection as pruritis, abnormal vaginal discharges, lower abdominal pain, dysuria or post-coital bleeding. Gynecological examination revealed that the infected cervix ranged from a clinically normal to a severely eroded with a hypertrophic cervical erosion and mucopurulent endocervical discharge. A consent was obtained from every patient.

Two cervical swabs were taken from each patient after cleaning the cervix with a dry cotton swab. Dacron swabs (Human) [Human for biochemicals and diagnostic mbH,.Max-Plank-Ring 21-D-65205 wiesbaden-Germany] were used for collecting cervical discharges from the endocervix and were preserved in sucrose phosphate saline (SP) *Chlamydia* transport medium (Vircell) [Vircell manufacture,S.L.Plaza Dominguez Ortiz 1Poligono industrial Dos de Octobre18320 Santa Fe (Granda) Spain]. One swab was shaken on a vortex mixer then it was discarded after pressing it against the tube wall. The contents of the tube were used for direct fluorescent antigen detection (DFA), and ELISA for antigen detection and the second swab was shaken on a vortex mixer then it was removed after pressing against the tube wall. Then, the tube was divided into two aliquots one for tissue culture and the other was preserved at -80°C until tested by PCR.

DFA

The specimens were centrifuged at 300 rpm for 10 min and the supernatant was discarded. The tubes were gently tapped to loosen the cells, then washed twice by PBS and 500 ul was added to each tube to yield an opalescent cell suspension. In each well of teflon-coated slides, 35 µl of cell suspension were delivered and examined under the microscope for presence of at least 5 epithelial cells /HPF which were considered adequate. The slides were dried and then fixed in cold acetone for 10 min. The slides were then stained by DFA (Vircell manufacture, S.L. Plaza Dominguez Ortiz 1Poligono industrial Dos de Octobre18320 Santa Fe (Granda)

Spain). The fluorescein isothiocyanate FITC-labelled monoclonal antibody (25 µl) against major outer membrane protein (MOMP) was added to each well and to that of positive and negative controls and incubated at 37°C for 30 min, washed with PBS for 10 min. Glycerin was added to each well and the slides were examined under the fluorescent microscope at 400 x. The sample was considered positive when the characteristic apple-green fluorescence inclusions were detected.

Tissue culture

McCoy cell line (Vircell) was used to support the *Chlamydial* growth. All steps were performed in class II biological safety cabinet (HEPA filter). The medium was removed from the flask and the monolayer cells were washed three times with 5 ml of sterile prewarmed PBS. Two mls of pre-warmed sterile trypsin-EDTA mixture (0.05% trypsin and 0.02% EDTA in PBS) were added to dissociate the cells from the flask. After 5 min, the cell monolayer became opaque and started to detach. The flask was tapped from time to time and when cells were completely detached, the trypsin was poured off. 10 mls of sterile growth medium (RPMI) supplemented with 10% fetal calf serum (FCS), gentamicin 50 mg/L, vancomycin 100 mg/L and amphotricin B 50 mg/L were added to each flask [Applichem Gmbh Ottoweg 4 D-64291 Darmstadt Germany]. The cells were then distributed on tissue culture plates (0.5 ml of cell suspension for each well), then incubated at 35°C in 5%CO_2 until complete monolayer sheet was formed within 72 h.

One of the two previously preserved aliquots was brought to room temperature then centrifuged with sterile glass beads to disrupt the epithelial cells and release of *Chlamydiae* elementary bodies. 200 µl of the specimen were inoculated into tissue culture plate wells after the medium was decanted. For each set of specimens, 2 non inoculated tissue culture wells were used as a negative control and 1 for positive control (L2 *Chlamydia* strain from Vircell). The inoculated tissue culture plates were centrifuged using ALC 4237R refrigerated centrifuge (from Lab X : P.O 478 Bay Street Midland, ON, Canada) for 1/2 h at 700 g then 0.5 ml maintenance medium supplemented with FCS (5%, L-glutamin 2.2 g/L , vancomycin 100 mg/L, gentamicin 50 mg/L, and amphotricin B 50 mg/L) was added to each well. The plates were incubated at 35°C in 5% CO_2 for 2 h to allow the adsorption of *Chlamydia*. After that the plates were incubated at 35°C in 5% CO_2 for 72 h. The cells were harvested by scraping (Kaye et al., 2005) and stained by immunofluorescent technique.

ELISA

It was based on the principle of a one step enzyme immunoassay using mouse IgG monoclonal antibody for detection of Chlamydia lipopolysaccharide antigen (LPS) (Mast, Nova Tec Immundiagnostica GmbH Waldstrasse 23 A 6 D-63128 Dietzenbach).

PCR

The preserved aliquots of samples were allowed to reach room temperature, and then centrifuged at 10,000 rpm for 15 min. The supernatant was poured off. *Chlamydia* DNA was extracted using QIAamp DNA kit "Qiagen".

A primer pair was selected from the conserved region of MOMP gene of *C. trachomatis*. The sequences of these oligonucleotide primers from 5' to 3' are as follows: Sense: 5' GCC GCT TTG AGT TCT GCT TCC 3'. Antisense: 5' GTC GAA AAC AAA GTC ACC ATA GTA 3' (Mania-Pramanik et al., 2006). PCR was done in a total volume of 50 µL. The final mixture contained primers (0.5 mM each), 0.2 mM dNTPs, PCR buffer (10 mM Tris-HCl, pH 9, 50 mM

Table 1. Identification methods of *Chlamydia trachomatis* (70 cases).

Test	No of +ve cases	%
McCoy cell culture	16	22.8
DFA	13	18.6
ELISA	28	40
PCR	18	25.7

Table 2. Comparison of DFA to McCoy cell culture for diagnosis of *C. trachomatis.*

		Tissue culture					Sen.	Sp.	Acc.	PPV	NPV	P
		-ve		+ve		Total						
		No	%	No	%	No	%	%	%	%	%	%	
DFA	-ve	52	96.3	5	31.3	57	81.4	68.75	96.3	90	84.62	91.23	0.000
	+ve	2	3.7	11	68.8	13	18.6						
	Total	54	100	16	100	70	100						

Sen. = Sensitivity, Sp. = Specificity, ACC. = Accuracy, PPV = Positive predictive value and NPV = Negative predictive value.

Table 3. ELISA compared to McCoy cell culture for the diagnosis of *Chlamydia trachomatis* infection.

		Tissue culture					Sen.	Sp.	Acc.	PPV	NPV	P
		-ve		+ve		Total						
		No	%	No	%	No	%	%	%	%	%	%	
ELISA	-ve	35	64.8	7	43.8	42	60	56.25	64.81	62.86	32.14	83.33	0.131
	+ve	19	35.2	9	56.3	28	40						
	Total	54	100	16	100	70	100						

KCL, 1.5 mM MgCL2) and 1.25 U of Taq polymerase. From each specimen, 10 μl of DNA was used for amplification. PCR tubes were processed in the thermal cycler for 35 cycles of amplification. Different cycles consisted of sequential incubations at 94°C for 1 min for denaturation, 60°C for 1 min for annealing, and 72°C for 2 min for DNA chain extension. At the end of the PCR cycles, the specimens were kept for 7 min at 72°C to complete the extension of the DNA chain. The PCR products were loaded onto 2% agarose gel stained with ethidium bromide for visualization after electrophoresis. Specific bands were detected at 180 pb.

RESULTS

McCoy cell line was used for isolation of *C. trachomatis* and stained by fluorescent monoclonal Ab for inclusion bodies. It was positive in 22.8% of cases and was considered as gold standard technique. Non culture based techniques were performed including DFA, ELISA, and PCR. They were positive in 18.6, 40 and 25.7% of cases respectively (Table 1).

By comparing DFA method to the culture method (Table 2), 11/70 of cases were positive by both techniques and 52 were negative cases by them. The

sensitivity of DFA was (68.75%), specificity (96.3%) with PPV (84.62%) and NPV (91.2%). There was statistically significant association between both (P = 0.000).

Out of 70 cases, 28 (40%) were positive by ELISA antigen detection, 9 of them were positive by both techniques (Table 3). Antigen detection by ELISA had sensitivity (56.25%), specificity (64.81%), NPV (83.33%) and PPV (32.14%). There was no statistically significant association between both techniques (P = 0.131).

Eighteen cases (of 70) were positive by PCR, 13 of them were positive by both techniques (Table 4). PCR sensitivity was 81.25%, its specificity was 90.74%, NPV was 94.23% and PPV was 72.22%. There was highly statistically significant association between both techniques (P = 0.000).

DISCUSSION

Confirmation of *chlamydial* infection usually depends on taking an appropriate specimen from the patient followed by direct detection of the organism using a suitable

Table 4. Value of PCR compared to McCoy cell culture in diagnosing genital *Chlamydia trachomatis.*

		Tissue culture						Sen.	Sp.	Acc.	PPV	NPV	P
		-ve		+ve		Total							
		No	%	No	%	No	%	%	%	%	%	%	
	-ve	49	90.7	3	18.8	52	74.3	81.25	90.74	88.57	72.22	94.23	0.000
PCR	+ve	5	9.3	13	81.3	18	25.7						
	Total	54	100	16	100	70	100						

laboratory-based diagnostic test. The important progress in laboratory diagnosis of chlamydial infection includes the development of non-viability-dependent tests, which are gradually being superseded by methods to detect chlamydial nucleic acid by direct hybridization (Boyadzhyan et al., 2004).

In this work, 70 cases were examined for *C. trachomatis* by different techniques. McCoy cell culture detected by immuno fluorescent technique was positive in 16 cases and we used it as a gold standard technique.

Direct fluorescent antigen detection (DFA) was compared to McCoy cell culture. Thirteen cases (18.6%) were positive by DFA. The sensitivity of DFA (68.75%) was lower than its specificity (96.3%). This is in agreement with Pate et al. (1998), who reported that DFA sensitivity (59.5%) was lower than specificity (99.6%). Similarly, Thejls et al. (1994), reported that DFA sensitivity was (66.7%) and specificity was (100%).

Higher specificity (96.3%) of DFA was in agreement with Fredlund et al. (2004), who stated that DFA for *C. trachomatis* specific anti-MOMP monoclonal antibody is considered to be highly specific. As regard NPV and PPV of DFA, there was a wide variation between the studies. NPV was (91.3%), while PPV was (84.62%). This is in parallel to Thejls et al. (1994) who reported that the NPV and PPV of DFA were (98.3%) and (91.7%) respectively. Pate et al. (1998) reported that PPV was (96.2%) while NPV was (93.9%).

The main disadvantage of DFA is that it is highly subjective (Lauderdale et al., 1999). DFA can give a quick turnaround time, while its sensitivity and specificity are dependent on the expertise of the personnel. It could detect both viable and non-viable organisms (Loeffelholz et al., 1992).

Value of direct antigen detection by ELISA in diagnosing *C. trachomatis* compared to culture was illustrated in Table 3. It was positive in 28 (40%) cases. The sensitivity was (56.25%), specificity (64.81%), NPV (83.33%) and PPV (32.14%).

Similar results were reported by some authors who found that antigen detection by ELISA had a low sensitivity of (48%) while specificity was (92.9%.) (Mahilum-Tapay et al., 2007).

Sensitivity of ELISA varies from 65 to 75% Chernesky (2005). On the other hand, some authors reported that sensitivity of ELISA was 50 and 58%, respectively and

the specificity was 100% by both studies (Malik et al., 2006; Mania-Pramanik et al., 2006). However, it was stated that in low prevalence populations the positive predictive value decreases rapidly with specificity which is a mathematical relationship (Dierksheide, 1987).

In our study, the NPV of ELISA was higher (83.33%) than its PPV (32.14%). Malik et al. (2006), found that the accuracy of antigen detection by ELISA was 82.7%. PPV was (66.6%) and NPV was (85.85%). They reported that in the absence of requisite infrastructure and skills for culture and for DFA, ELISA can play a significant role in screening for *C. trachomatis.*

In this study, DFA was more specific than ELISA and this could be explained on the basis that the test detects MOMP of *C. trachomatis* (Tables 2 and 3). It was reported that DFA found to be a specific test as it detects the MOMP antigen of *C. trachomatis* (Mahilum-Tapay et al., 2007).

There are many commercially available EIA tests on the market for detecting *C. trachomatis* infection. They detect Chlamydia LPS with a monoclonal or polyclonal antibody that has been labelled with an enzyme (Loeffelholzet al., 1992). LPS is more abundant and more soluble than MOMP but less specific (Pate et al., 1998). The diagnostic efficacy of PCR was compared to McCoy cell culture (Table 4). The sensitivity was 81.25%, specificity (90.74%), NPV (94.23%) and PPV (72.22%). There is highly statistically significant association between both techniques in diagnosing genital Chlamydia infection (P = 0.000).

Diagnosis of genital *C. trachomatis* infection by PCR showed a sensitivity, specificity, PPV and NPV 92.9, 96.2, 79.6 and 98.8%, respectively (Fredlund et al., 2004). Similar sensitivity (91%) but higher specificity (100%) were reported (Jalal et al., 2006). Tan and Chan (2005) reported that, the sensitivity of PCR was 100% and specificity was 98 to 100%. This high sensitivity might be explained on the basis that, they use the automated system "Cobas Amplicor", target difference and the use of 5 pools of swabs from each patient.

Results of PCR in our study were significantly associated with the results of McCloy cell culture (P = 0.000). This could be similar to findings recorded by Bauwens et al. (1993) and Pate et al. (1998) who found that there was a good correlation between PCR and culture for diagnosing genital *C. trachomatis* infection.

DFA is more sensitive than ELISA, however, it is not suitable for work on a wide scale as it is subjective and very labour intensive (Thejls et al., 1994). The CDC recommended molecular biologic technique for confirmation of positive results (Tong et al., 1997). Bébéar and de Barbeyrac (2009) concluded that the diagnosis of *C. trachomatis* is best made by using nucleic acid amplification tests, because they perform well and do not require invasive procedures for specimen collection.

In summary, McCoy cell culture assay is the most reliable test, but tedious, time consuming, costly and inconvenient for diagnosis *C. trachomatis* specially in developing countries. Antigen detection by ELISA was of high NPV, and low PPV (which may be due to low prevalence populations). DFA was of high specificity (96.3%) and PCR was of the high sensitivity (81.25%). Combination of both tests could optimizes diagnosis of female genital Chlamydia. It could be recommended that wide scale studies and new tests should be evaluated for an expanded gold standard based on multiple tests each with proven high sensitivity and specificity. In addition, different females in different localities in Egypt should be evaluated to assess combination of tests to realize the most simple, rapid and accurate technique that facilitate diagnosis and epidemiological studies for prevalence rates of *C. trachomatis* infection in Egyptian females.

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