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COMPARISON BETWEEN THREE DIFFERENT LABORATORY METHODS IN DIAGNOSIS OF CHLAMYDIA TRACHOMATIS

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ABSTRACT

This study was conducted on 40 non pregnant women suffering from lower genital discomfort for detection of chlamydia trachomatis (C. trachomatis) from their cervices by three different laboratory tests (Giemsa stain, Direct immunofluorescent antibody "DFA" stain, and culture on BGM cells). We found that 62.5% of young females (16 - 25 years) had C. trachomatis while older ones (26 - 35 years and 36 - 54 years) had less infections (25% and 12.5% respectively). 75% of women from rural areas had C. trachomatis while 25% of those from urban areas had C. trachomatis. As regards to the symptoms and signs, 50% of women with cervicitis had C. trachomatis. As regards to the laboratory methods, 8 cases (20%) were positive by Giemsa stain, 10 cases (25%) were positive by DFA, and 13 cases (32.3%) were positive by culture method. As regards to sensitivity and specificity to culture method, Giemsa stain was 61.5%, 100%, and DFA were 61.5% and 92.5 respectively. We conclude that, tissue culture method is most sensitive and specific method and considered to be the gold standard test for detection of C. trachomatis, however, processing time and cost have decreased its use in clinical practice.

INTRODUCTION

Chlamydia trachomatis (C. trachomatis) is among the most common world wide sexually transmitted bacteria (McCormack et al., 1999).

C. trachomatis had been isolated

from female genital tract and most infections involve the cervix. The infection may be clinically inappearent or may result in severe cervicitis and often superimposed on cervical erosion (Howell et al., 1999).

As, C. trachomatis is the leading cause of nongonococcal urethritis and cervicitis in women, and because of the recent increase in the numbers of new cases and severe consequences, there is an urgent demand for sensitive and specific rapid diagnostic methods for its detection (Deak et al., 1997).

Several methods were used for detection of C. rachomatis, including direct methods; as Giemsa stain, culture, and direct immuno-fluorescent staining, or indirect methods as detection of its specific antibodies by enzyme linked immunosorbent assay (ELISA) (Compbell et al., 1993). Dean et al., (1998) stated that all of new techniques, are costly, require skilled personnel, and are not available to all laboratories, also, they are still considered to be less sensitive than isolation techniques for detection of C.trachomatis.

Aim of the work :

This study aimed to compare between three different laboratory methods (Giemsa stain, direct immunofluorescent staining and tissue culture) in detection of C. trachomatis in non-pregnant women suffering from lower genital tract discomfort.

Subjects and methods :

40 non-pregnant women (aged from 16 to 54 years) suffering from lower genital discomfort, i.e. irritation, soreness, and lower backache were selected from Out-Patients Clinics of Gynaecology and Obstetric Department, Zagazig Faculty of Medicine.

All selected women did not receive any antimicrobial therapy either locally or systemically during the previous week of study.

A full gynecological examination , and 2 endocervical dacron swabs (supplied from ORION DIAGNOSTIC, FINLAND) were taken from each woman.

N.B. Each swab was enrolled firmly in the cervix to desquamate enough epithelial cells.

(1) The first swab was rolled firmly

over a glass slide for Giemsa stain and its other face was rolled over a single well of Tephlon slide (N.B.: if the slide or well did not appear opaque, the rolling was repeated again).

(a) Detection of chlamydial elementary bodies by Giemsa stain was done (according to Collee et al., 1989).Slides were examined by oil immersion lens, positive cases showed intra-cytoplasmic inclusions appeared as small and purple areas around the pink nucleus.

(b) Detection of chlamydial elementary bodies was done by direct immunofluorescence antibody (DFA) staining. Kit was supplied from (Pathfinder, Direct Antigen Detection System, Chalets Diagnostics, Chaska). Slides were examined using immuno-fluorescent microscope (Zeiss, Japan) using oil immersion lens. Positive specimens showed greenish elementary bodies contrasted by reddish black background.

(2)The second swab was put in a sterile bottle containing chlamydia transport media (prepared according Bailey and Scott, 1986) and 2 glass beeds and kept in ice bags till transported to the laboratory.

Bottles were centrifuged at 10,000 rpm speed for 10 minutes and the deposits were cultured on BGM cells (Buffalo-Green-Monkey continuous kidney cell) supplied from Vac-Sera Institute. Cultures were done according to Baron and Fineold, (1990) ,stained by Iodine stain and examined after 5 days. In positive cases, BGM cells appeared as pale yellow sheet with dark brown intra-cytoplasmic elementary bodies.

RESULTS

Our results are illustrated in tables 1 - 4.

Table (1) : Comparison between the positive results of C. trachomatis by different laboratory tests regarding to age, residence, symptoms and signs

Parameter	Giemsa stain (8)				DFA (10)				Tissue culture (13)			
	No.	%	X^2	P	No.	%	X^2	P	No.	%	X^2	P
Age :												
16 - 25 years	5	62.5			6	60			9	69.2		
26 - 35 years	2	25	1.01	>0.05	3	30	0.63	>0.05	2	15.4	0.93	>0.05
36 - 54 years	1	12.5			1	10			2	15.4		
Residence :												
Rural	6	75	6.02	>0.05	7	70	0.91	>0.05	9	69.2	0.7	>0.05
Urban	2	25			3	30			4	30.8		
Symptoms and/or signs :												
- Discharge only	1	12.5			1	10			2	15.4		
- Irritation and soreness	1	18.5	1.05	>0.05	2	20	0.82	>0.05	2	15.4	1.01	>0.05
- Cervicitis	4	50			4	40			5	38.5		
- Cervical erosion	2	25			3	30			4	30.7		

DFA : Direct immuno-fluorescent antibody staining. P>0.05 : Non significant

Table (2) : Results of different laboratory tests regarding to diagnosis of C. trachomatis.

C. trachoma-	Giemsa stain (8)				DFA (10)				Tissue culture			
	No.	%	X^2	P	No.	%	X^2	P	No.	%	X^2	P
+ ve	8	s20			10	25	8.54	<0.005	13	32.5	8.69	<0.005
- ve	32	80	8.61	<0.005	30	75			27	67.5		
Total	40	100			40	100			40	100		

DFA : Direct immuno-fluorescent antibody staining. P<0.005 : Significant

Table (3) : Comparison between tissue culture and Giemsa stain regarding to the diagnosis of C. trachomatis.

Test		Tissue culture + ve	Tissue culture - ve	Total
Giemsa stain	+ ve	8	Zero	8
	- ve	5	27	32
Total		13	27	40

Sensitivity : 61.5% **Specificity** : 100% **+ve PV** : 100% **-ve PV** : 84%

Table (4) : Comparison between tissue culture and DFA stain regarding to diagnosis of C. trachomatis.

Test		Tissue culture + ve	Tissue culture - ve	Total
DFA	+ ve	8	2	10
	- ve	5	25	30
Total		13	27	40

DFA : direct immunofluoresence antibody stain.

Sensitivity : 61.5% **Specificity :** 92.5% **+ve PV :** 80% **-ve PV :** 83.3%

DISCUSSION

C. trachomatis infection has been associated with a wide spectrum of syndromes ranging from occular to genital tract diseases. In industrialized countries, genital infections are now recognized as a major public-health problem (McCormack et al., 1999).

Giemsa stain was used for detection of C. trachomatis from endocervical swabs. However, certain problems were encountered, regarding cervical swabs, many of the cells were squamous and inclusions are found only in columnar cells, this was the case especially in specimens where the patient had a severe cervical erosion where bleeding can occur while taking a perfect sample. Also, in some others, there were a lot of bacterial debris and mucous plugs in specimens that mislead our results.

Our results showed that , 20% of the studied females were chlamydia positive by Giemsa stain . The difference of C. trachomatis detected by Giemsa stain and other techniques may be due to the problems discussed before.

Our results are supported by Massoud et al., (1991) who found only 19.6% of +ve cases of C. trachomatis by Giemsa stain and stated that, the failure to demonstrate inclusions in the stained smears may be due to its hidding in densely stained clumps of cells.

Compared with tissue culture, sensitivity of Giemsa stain was 61.5% and specificity was 100%, with 100% positive predictive value and 84% negative predictive value. These results are in agreement with Deak et al., (1997) who stated that Giemsa stain can be used for diagnosing C. trachomatis infection in high prevalence setting such as Sexually Transmitted Diseases Clinics. Giemsa stain appears to be a rapid screening test , but needs careful training in reading smears, that will be critical in the resulting sensitivity and specificity of the test.

It is suspected that, patients who are culture positive and Giemsa negative may be due to sampling error, that is, none of the small number of elementary bodies on the swab are deposited upon the slide which may be the cause in the five patients positive by tissue culture and negative by Giemsa stain. A small number of elementary bodies on the slide are hard-

er to be recognized than large ones (Boisvert et al., 1993).

Testing for the presence of C. trachomatis in clinical specimens has been revolutionized by the development of monoclonal antibodies to C. trachomatis, these antibodies allow the visual detection of elementary bodies in different pathological specimens. This method is rapid and does not depend on cold chain transportation system, and also, is ideal for testing few specimens, particularly from clinics remote from the laboratory (Orndorff, 1991).

As regards to DFA method, our study revealed that 25% of women were chlamydia positive. This may be due to good staining as separate elementary bodies bind well with the specific labelled antibodies.

Our results are nearly similar to Dean et al., (1998) who detected C.trachomatis in 28.6% of their patients by DFA method.

Comparing the results of DFA and tissue culture in our study, 8 cases were positive by both tests while 2 cases were positive by DFA only and 5 cases were positive by culture only. It is suspected that 5 patients who are culture positive and DFA negative may be due to sampling error, non of the small number of elementary bodies on the swab are depositied upon the slide. The 5 positive cases by culture only and negative by DFA may be due to the absence of the specific antigen of C. trachomatis at the time of staining. This comment is supported by Szarewski et al., (1991) who concluded that using of specific epitope of C. trachomatis will overcome this problem. The two positive patients by DFA and negative by tissue culture may be due to presence of toxic factors that interfere with the growth cycle of the organism, producing false negative results upon culturing.

Compared with tissue culture, the performance of DFA test was as follows : sensitivity of 61.5% and specificity of 92.8%, with 80% positive predictive value, and 83.3% negative predictive value.

Our comment can be supported by Orndorff (1991), who suggested that DFA test may be even more sensitive than the chlamydia culture and can detect low levels of infection and stated that, DFA detects chlamydial anti-

gen irrespective of the viability of the organism, while culture isolation requires living organism.

Schubiner et al., 1992, found the performance of DFA staining and cell culture have produced varying sensitivity and specificity resemblance, due to differences in: (a) The sensitivity of the cell culture method, (b) The patient population examined, (c) Various cut off points used for the number of elementary bodies required to consider the test positive.

Cell culture isolation of C. trachomatis remains the most sensitive and specific method for identifying C. trachomatis (Boisvert et al., 1993).

As regards to results of cell culture technique, our study revealed 13 positive cases (32.5%). These results are similar to that of Ismail et al., (1992) who used the same type of cells (BGM) with the same technique in isolation of C. trachomatis.

Our results of culture are supported by (Deak et al., 1997) who stated that cell culture has traditionally been the gold standard test for laboratory diagnosis of C. trachomatis infection. Because of its high specificity, cell culture is also a reliable research tool. However, processing time and cost have decreased its use in clinical practice.

It is difficult to estimate the true prevalence of chlamydial infection in lower genital tract infection in women. The prevalence of C. trachomatis infection amongst the female population is high, but varies greatly in different groups relative to age, and pattern of sexual behaviour (Beebe et al., 1999).

In our study as regard to age, high prevalence of C. trachomatis, was recorded in young age group 16-25 years (62.5%) and this reveals that chlamydial carriage rate is related to the sexually active young women. Our findings were supported by Cloud et al., (1993) and Cohen et al., (1999) who stated that C. trachomatis incidence and prevalence have increased to epidemic proportion in young sexually active women. Also, Rolfs, (1999) found that 28.7% of young adolescent girls were C.trachomatis positive, and suggested that, uncontrolled sexual activity is responsible for this results, or that possible relapse of an initial chlamydial infection.

As regards to residence, our study showed a higher incidence of C. trachomatis infection in the rural women (75%) rather than the urban women (25%).

Our findings were in agreement with that of Beebe et al., (1999) and Cohen et al., (1999) who stated that C. trachomatis infections increased in bad sanitary and hygenic conditions as well as in low standards of living . Also, Rolfs (1999) found that 33.6% of Chlamydia positive women were from rural areas while only 10.9% of positive women were from urban areas, and stated that sex education is essential for controlling genital chlamydial infection, this supports our results.

C. trachomatis may act as a primary pathogen causing acute clinical disease, but an equilibrium is often reached between host and parasite which leads to prolonged inapparent infection. In these subclinical infections ; multiplication of the organism is held in check by host defenses, but this balance may be disturbed by various factors, so that active replication of the organism occurs followed by an exacerbation of disease (Heikel et al., 1999).

In out study, women who were complaining of lower genital tract symptoms and/or signs that having cervicitis were chlamydia positive in 50% and 25% with cervical erosion were chlamydia positive, discharge only 12.5% irritation and soreness 18.5% (Nugent and Hillier, 1992).

Thomas et al., (1998) reported that although infected women with C. trachomatis are usually asymptomatic, the organism is far from benign. Sequential culturing of untreated women has demonstrated that chlamydial infection may persist for weeks or months without development of symptoms.

This is supported by Sales et al., (1998) who stated that abnormal cervix (cervicitis or erosion) is a term which simply means extension of the columnar epithelium into the area normally covered with squamous epithelium. This might increase the susceptibility of chalmydiae since they can not infect squamous cells. Also, Howell et al, (1999) found that mucopurulent cervicitis may be an indicator of increased risk for C. trachomatis infection and stated that most females with cervicitis have C. trachomatis infection, and these females responded

with complete resolution of their disease after a two week course of treatment.

We can conclude that, tissue culture technique for detection of C. trachomatis is better than Giemsa stain and DFA technique. Also, younger women from rural areas, and women had abnormal cervix were more positive for C. trachomatis infection.

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مقارنة بين ثلاث طرق معملية مختلفة لتشخيص الكلاميديا تراكوماتس

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*أقسام الميكروبيولوجي والمناعة - النساء والتوليد**

كلية طب بنها - كلية طب الزقازيق - جامعة الزقازيق

أجريت هذه الدراسة على ٤٠ سيدة غير حامل تعانى من آلام فى الجهاز التناسلى لتشخيص الكلاميديا تراكوماتس فى عنق الرحم بواسطة ثلاث طرق معملية مختلفة (صبغة جيمسا - صبغة الأميونوفلورسنت المباشرة والزرع على خلايا (بى جى إم) .

وقد وجد أن ٦٢٫٥٪ من السيدات صغار السن (١٦-٢٥سنة) لديهن ميكروب الكلاميديا تراكوماتس و ٧٥٪ من السيدات من المناطق الريفية لديهن ميكروب الكلاميديا تراكو ماتس و ٥٠٪ من السيدات اللاتى تعانى من إلتهاب فى عنق الرحم لديهن ميكروب الكلاميديا تراكوماتس.

ومن حيث النتائج المعملية وجد ميكروب الكلاميديا تراكوماتش فى ٢٠٪ من السيدات بواسطة صبغة جيمسا و ٢٥٪ بواسطة صبغة الأميونوفلورسنت المباشرة و ٣٢٫٥٪ بواسطة الزرع على خلايا (بى جى إم) ومن حيث الحساسية والخصوصية لطريقة الزرع وجد أن صبغة جيمسا كانت ٦١٫٥٪ و ١٠٠٪ وبالنسبة لصبغة اللأميونوفلورسنت وجد أنها ٦١٫٥٪ و ٩٢٫٥٪ على التوالى .

وقد وجد أن طريقة الزرع أكثر حساسية وخصوصية فى تشخيص ميكروب الكلاميديا تراكوماتس رغم أن الوقت والتكلفة تقلل نسبة إستخدامها عملياً .