Egyptian Journal of Medical Microbiology Volume 24 / No. 3 / July 2015 61-68

ORIGINAL ARTICLE

Tissue Culture versus PCR in Diagnosis of Female Genital Chlamydia Trachomatis Infection

[1]Ahmed M. El-Gazzar, [1]Waffa A. EL-Mosallamy, [1]Sherin M. Emam, [2]Ahmed Walid Anwar Morad and [1]Maysa S. Moustafa*

[1]Department of Medical Microbiology and Immunology, Faculty of Medicine, Benha University
[2]Department of Gynacology & Obstetrics, Faculty of Medicine, Benha University

Key words:

C.trachomatis, PCR , tissue culture

ABSTRACT

Background: *Chlamydia trachomatis (C.trachomatis)is the most prevalent sexually transmitted pathogen worldwide. It is common among sexually active young women.* ***Objectives:*** *Evaluate the sensitivity and specificity of tissue culture followed by antigen detection by immunoflurescencein comparison with nested PCR for diagnosis of genital C. trachomatis infection.* ***Methodology:*** *This study was carried out on 50 women, among those attending the Gynecology Outpatient Clinics of Benha University Hospital during the period from May 2014 to March 2015. The participants suffer from symptoms suggestive of genital C. trachomatis infection , their ages ranged from 20 to 39 years old (mean ± SD =29.80±4.647) .Two endocervical swabs were taken from each patient . One used for cytological examination, and the other used for tissue culture detected by , immunoflurescence as well as nested PCR.* ***Results:*** *The result of tissue culture detected by direct immunoflurecence revealed that out of 50 patient , 24 (48%) were positive for C.trachomatis and 26 (52%) were negative .Result of nested PCR revealed that out of 50 patient, 34 (68%) were positive and 24 (32%) were negative . The result of cytological examination revealed that out of 50 patient , 17 (34%) were positive and 33 (66%) were negative. The sensitivity, specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPP) of tissue culture for detection of C.trachomatis were 70.6%, 100%, 100% and 61.5% respectively. Roc curve of tissue culture revealed that it is considered to be a good test compared to PCR in diagnosis of genital C.trachomatis.* ***Conclusion****: The tissue culture as detected by immunoflurescence is a good test in relation to nested PCR in diagnosis of genital C.trachomatis.*

INTRODUCTION

C. trachomatis is an obligate intracellular human pathogen which is responsible for the most reported bacterial sexually transmitted disease worldwide. The prevalence of these infections can be different depending on the country and population type. The prevalence of *C. trachomatis* infection among sexually active women in developing countries is higher than developed countries[1].

Although, infection with this organism can be asymptomatic in up to 80% of women, it may give rise to urethral syndrome, salpingitis, pelvic inflammatory disease (PID), tubal factor infertility and chronic pelvic pain[2].

Corresponding Author:
Maysa S. Moustafa
Department of Medical Microbiology and Immunology, Faculty of Medicine, Benha University
E-mail: mysasaid@yahoo.com; **Tel** 01100434205

Infection of the female reproductive tract with *C. trachomatis* is one of the leading global causes oftubal factor infertility, and leading causes of female factor infertility . In order to reduce the rate of PID and prevent development of reproductive sequelae, early diagnosis and treatment of chlamydial infection can be of great importance[3].

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[4].

Since the prevalence of chlamydial diseases is on rise, development of sensitive, specific, and rapid methods to diagnose this infections is highly favored. Cell culture, cytological tests for the detection of cytoplasmic inclusion bodies, direct immunofluorescence (DFA), enzyme-linked

immunosorbent assay (ELISA), DNA hybridization techniques and polymerase chain reaction (PCR) are several laboratory methods which are used for the diagnosis of *C. trachomatis* [3].

The PCR has proved to be the gold standard to detect *C.trachomatis*. Nucleic acid amplification techniques such as PCR involve exponential amplification of well defined DNA targets, resulting in enhanced sensitivity of detection compared with the sensitivities of other laboratory tests [5].

The aim of the present study is to evaluate the sensitivity and specificity of tissue culture and cytological examination in comparison with nested PCR for diagnosis of genital *C. trachomatis* infection.

METHODOLOGY

Patients:

This study carried out on 50 women, among those attending the Gynecology Outpatient Clinic of Benha University Hospitalduring the period from May 2014 to March 2015. The participants suffered from symptoms suggestive of genital *C.trachomtis* infection (vaginal discharge, post-coital bleeding , inflamed or friable cervix, cervical erosion, urethritis, pelvic inflammatory disease, lower abdominal pain and reactive arthritis in sexually active women).The study protocol was approved by the Local Ethics Committee of the departments of obstetrics &gynecology, Benha faculty of medicine, Benha University. A consent was obtained from every patient.

Samples:

After cleaning the cervical canal with a dry cotton swab,two endocervical swabs were taken from each patient and used for collecting cervical discharges from the endocervix and were preserved in screw capped tubes containing *Chlamydia* transport media (VACERA) contained: Roswell Park Memorial Institute(RPMI) 1640 media (Sigma-Aldrich) with 2% fetal calf serum. One swab used for cytological examination and the other 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 that preserved at -80°C, one used for tissue culture and immunofluresence and the other was tested by nested PCR[6].

1. **Detection of inclusion bodies in smears stained by Giemsa (sigma)*:***

   Thin films of the collected specimens were spread on clean microscopic slides. The slides were air dried and fixed with methanol (methyl alcohol) for 2-3 minutes. Then the smears were allowed to air-dry. The slides were placed; smear downwards, in a Petri dish, supported on each slide by a thin piece of stick. The diluted stain was poured into the dish and cover with a lid. The smears were left to stain for 1-2 hours. The slide was washed from the stain and rinses the smear with buffer water.

   Microscopic examination: Slides were first examined by 40X objective lens to see the distribution of material and to select a suitable part of the smear to examine with the oil immersion lens [7].

2. **Tissue culture:**

   Hela cell linewere obtained from (VACSERA) institute at passage No/44 was used to support the Chlamydial growth. All steps were performed in class II biological safety cabinet with HEPA filter(AURA Mini-Germany)..The growth 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) 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. 30 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 . The cells were then distributed on tissue culture plates (3 ml of cell suspension for each well), then incubated at 35°C in 5% Co2 until complete monolayer sheet was formed within 72 h [6].

   One of the two endocervical swabs 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 . The plates were incubated at 35°C in 5% CO2 for 2 h to allow the adsorption of *Chlamydia*. To the inoculated tissue culture plates 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. After that the plates were incubated at 35°C in 5% CO2 for 72 h. The cells were harvested by scraping and fixed by acetone to be ready for staining by fluorescin-conjugated monoclonal antibody specific for *C. trachomatis* and examined by fluorescent microscope [8] .

3. **Identification of the growing C.trachomatis in tissue culture by direct immunoflurescentassay:**

   It was done as described by the manufacture (Omega Diagnostics).

   25µl *Chlamydia* Direct fluorescent antibody (DFA) Reagent was dispensed onto the fixed specimen smear and the positive control slide, covering the entire well area. The slides at 37ºC was incubated in a closed petri-dish with moisted piece of cotton for 30 minutes in the dark. The slides were not allowed to dry as this will

cause non-specific binding. The slides were gently rinsed in a bath of Phosphate Buffered Saline (PBS) for approximately 1 minute. The slides were drained and excess moisture around the wells was removed with absorbent tissue. One drop of Mounting Fluid was added to the well, covers slip was placed on top of the drop and air bubbles removed. The entire specimen was scanned using a fluorescence microscope under oil immersion at x600 to x1000 magnification.

Interpretation: Cells in the positive specimens will fluorescence apple green while uninfected cells will stain dull red due to the presence of Evan blue.

4. Nested PCR:

It was done as described by the manufacture (Thermo Scientific).

DNA extraction: Samples are digested with Proteinase K in the supplied Lysis Solution. The lysate is then mixed with ethanol and loaded onto the purification column, where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared Wash Buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Procedure: Specimens were thawed; 20 µL Proteinase K and 200 µL binding buffer were added to 200 µL of samples, after mixing, they were incubated at 60°C for 20 minutes. 100 µL isopropanol was added to mixtures. Lysates were centrifuged at 8000 rpm for 1 minute; washing buffer 1 and then 2 were added to tubes, respectively. After centrifuge, 200 µL elution buffer was added and centrifuged at 8000 rpm for 1 minute, finally, Eluted genomic DNA stored at -20°C for for further analysis.

DNA amplification: Momp gene was amplified by nested PCR(two sets of amplification reaction). The first specific primer set was:

(Chlamydia 1 F- 6) = 5 ('GGACAAATCGTATCTCGG-3'), and

(Chlamydia 1 R- 6)=(5'GAAACCAACTCTACGCTG-3)'.

The second primer set was (Chlamydia 2 F- 6) =(5' ATTGCTTGAGCGTATAAAGG-3'), and (Chlamydia 2 R-6)=(5'TGCTATAATCACGAAATTAC-3').

The amplification was carried out in thermal cycler (Biometra, Germany). The following components were added to each 50µl reaction tube at room temperature: 25µl Maxima Hot Start PCR Master Mix (2X) ,2.5 µl forward primer, 2.5 ml reverse primer , 5 ml template DNA, and 15 ml nuclease free water. First set of reaction using the first primer set, was performed as follow: Denaturation was performed at 95°C for 4 minutes. Followed by 35 cycles (95°C for 30 seconds, 49°C for 30 seconds and 72°C for 31 seconds) and 72°C for 5 minutes. The products of the 1st set used as template DNA for the second set .Second set of reaction using the second primer set, was performed as the first set except that the annealing temperature was 51°C and the extension time was 15 sec . PCR products were separated by 2% agarose gel electrophoresis and were visualized by ethedium bromide staining. The amplified product of first set was 517 bp and the amplified product of second set was 250 bp [9].

RESULTS

Result of nested PCR revealed that out of 50 patient , 34 (68%) were positive and 24 (32%) were negative Detection of nested PCR results by gel electrophoresis is shown in figure 1. The results of the tissue culture followed by immunoflurescence for antigen detection and cytological examination as regarding specificity and sensitivity were evaluated in relation to nested PCR results. The result of tissue culture for *C.trachomatis* detection by direct immunoflurecence revealed that out of 50 patient , 24 (48%) were positive for *C.trachomatis* and 26 (52%) were negative .Antigen detection of growing Chlamydia in tissue culture by direct immunoflurescenceis shown in figure 2. The result of cytological examination revealed that out of 50 patient, 17 (34%) gave were positive and 33 (66%) were negative. Results of study revealed that, The sensitivity, specificity, Positive predictive value (PPV), and negative predictive value (NPP) of tissue culture for detection of *C.trachomatis* were 70.6%, 100%, 100% and 61.5% respectively as shown in table 1. Roc curve of tissue culture revealed that it is considered to be a good test compared to nested PCR in diagnosis of genital *C.trachomatis* as shown in figure 3. The sensitivity, specificity Positive predictive value (PPV), and negative predictive value (NPP) of cytological examination was 50%, 100%, 100% and 48.5% respectively as shown in table 2. Roc curve of cytological examination revealed that it is considered to be a fair test compared to nested PCR in diagnosis of genital *C.trachomatis* as shown in figure 4 .

The analysis of personal history and presenting symptoms of the studied patients in comparison with positive and negative *C. trachomatis* cases as detected by nested PCR reveals that , The differences between positive and negative results were significant as regarding abnormal vaginal discharge and cervical erosion and were insignificant regarding age group, menstrual history, history of abortion fertility condition, contraceptive method, post-coital bleeding and associated arthritis as shown in table 3 .

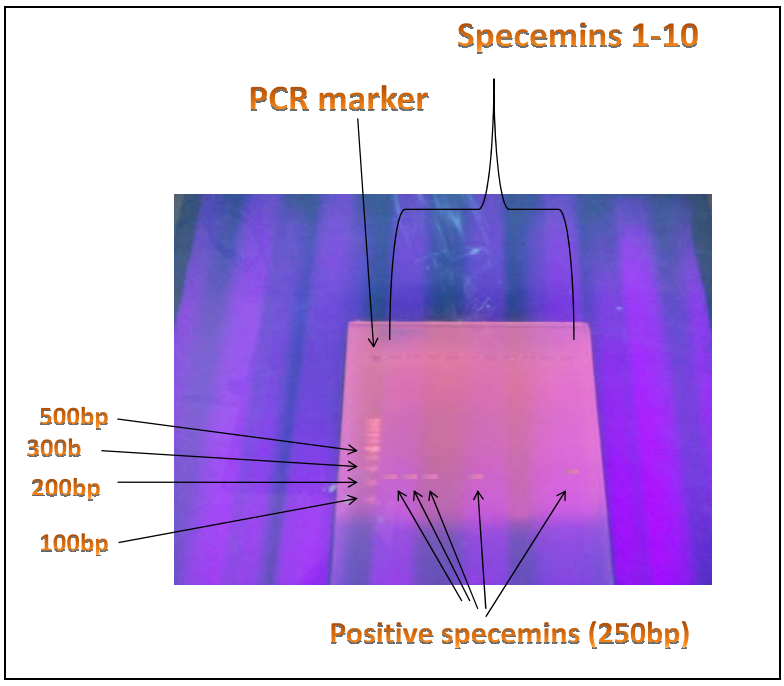

Fig. 1: Shows detection of nested PCR results by gel electrophoresis for detection of C. trachomatis in endocervical swab

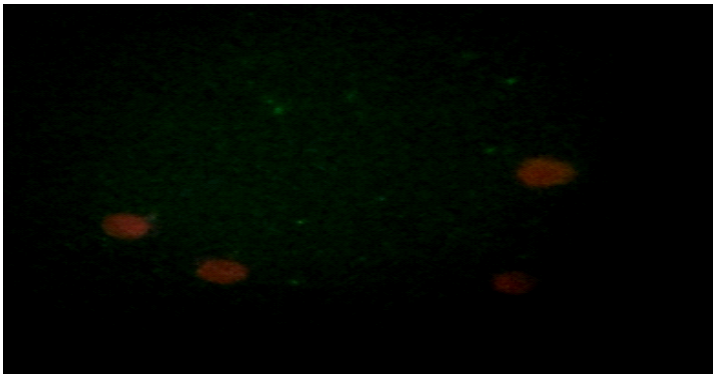

Fig. 2: Shows antigen detection of growing C. trachomatis in tissue culture by direct immunoflurescence .

Table 1: Comparison between the results of tissue culture followed by immunoflurescence for antigen detection and nested PCR in diagnosis of C.trachomatis in endocervical swab:

Nested PCR / Tissue culture	Positive		Negative		Total		p-value
	No.	%	No.	%	No.	%	
Positive	24	70.6%	0	0.0%	24	48.0%	<0.001
Negative	10	29.4%	16	100.0%	26	52.0%	
Total	34	100.0%	16	100.0%	50	100.0%	

Sensitivity= 70.6% Specificity=100%
PPV=100% NPV=61.5%
P value <0.001 (highly significant)

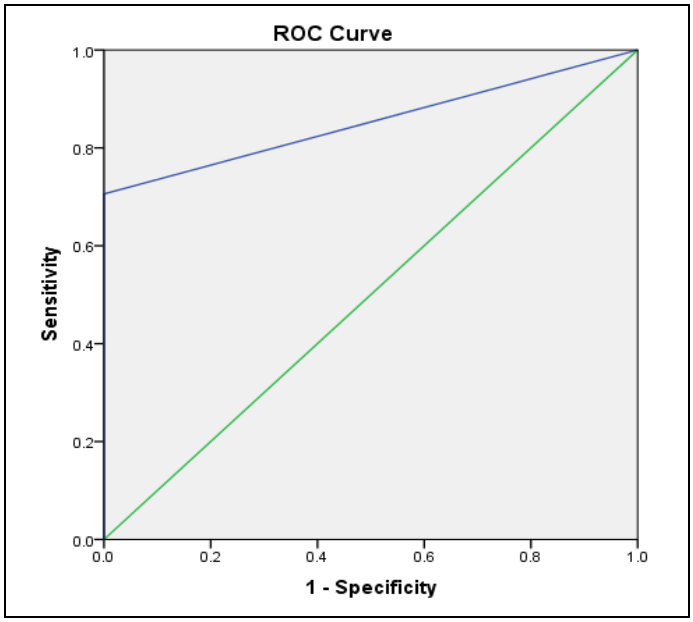

Fig. 3: Receiver operating characteristic (ROC) curve for tissue culture followed by immunoflurescence for detection of C.trachomatis in endocervical swab.

Table 2: Comparison between results of cytological examination (CEX) and nested PCRin diagnosis of C.trachomatis in endocervical swab :

Nested PCR / CEX	Positive		Negative		Total		p-value
	No.	%	No.	%	No.	%	
Positive	17	50.0%	0	0.0%	17	34.0%	<0.001
Negative	17	50.0%	16	100.0%	33	66.0%	
Total	34	100.0%	16	100.0%	50	100.0%	

Sensitivity= 50% Specificity=100%
PPV=100% NPV=48.5%
P value <0.001 (highly significant).

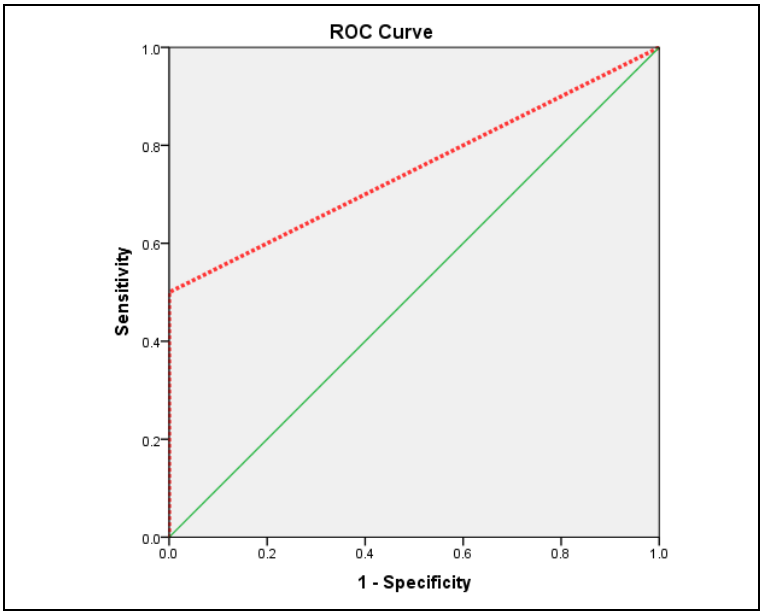

Fig. 4: Receiver operating characteristic (ROC) curve for cytological examination in diagnosis of C.trachomatis in endocervical swab.

Table 3: Comparison of positive and negative C. trachomatis cases detected by nested PCR cases as regarding personal history and presenting symptoms:

	P value
Age group	>0.05
Menstrual history	>0.05
History of abortion	>0.05
Fertility condition	>0.05
Contraceptive method	>0.05
Abnormal vaginal discharge	<0.05
Post-coital bleeding	>0.05
Cervical erosion	<0.05
Associated arthritis	>0.05

DISCUSSION

C.Trachomatis is the most prevalent sexually transmitted pathogen worldwide. It is common among sexually active young women[10]**.** The aim of the present study is to evaluate the sensitivity and specificity of tissue culture and cytological examination in comparisonwith PCR for diagnosis of genital *C. trachomatis* infection.

As regard the results of tissue culture in comparison with nested PCR results in diagnosis of genital *C.trachomtis*, out of (34) positive cases of PCR: It reveals that out of 34 (100%) patients positive for PCR , 24 (70.6%) patients was positive for tissue culture . And out of 16 patient negative for nested PCR , (0 %) patients was positive for tissue culture . The sensitivity, specificity , positive predictive value (PPV) and negative predictive value (NPV) of the tissue culture in relation to PCR were 70.6% , 100% , 100% , 61.5% respectively .ROC curve analysis revealed that tissue culture is a good test in relation to PCR.In Iran, Hajikhani et al**.**[3] performed a study on women with tubal factor infertility, attending Avicenna Infertility clinic . They reported that, the sensitivity of tissue culture was 75%. and specificity was 100%. PPV was =100%, but NPV was =91.8% , and their results are in line of our results. Another study conducted by Agha et al. [6] on females attending Outpatient Gynecology Clinic, Mansoura University Hospital, Egypt, reported that the sensitivity and specificity of tissue culture were 72.2% and 94.2% respectively which are in agreement of our results. Our results are also in line with Cheng et al. [11] who conducted a comparative study between PCR and culturing *Chlamydia* on patients attending the Jefferson County Department of Health STD clinic in Birmingham have reported that the sensitivity and specificity of tissue culture were 80 % and >95 % respectively . In Washington, a report study performed by Johnson et al.[12] in the National Center for HIV, STD, and TB Prevention,. They reported a high specificity of *Chlamydia* tissue culture, and 74.4% sensitivity which agreed with our results. In contrast of our results, a study performed by Bachmann et al. [13] on individuals attending a sexually transmitted disease clinic and three HIV clinics who gave a history of anal intercourse or were women at high risk for *Neisseria gonorrhoeae* or *Chlamydia trachomatis* infections . Rectal swab specimens were tested using culture and commercial nucleic acid amplification tests (NAATs), employing transcription-mediated amplification (TMA), strand displacement amplification (SDA), and PCR amplification**.** Test performance was evaluated using a rotating standard by which patients were classified as infected if either two or three comparator tests were positive**.** They reported that the sensitivity of tissue culture was ranged from (36.1% to 45.7%). These results is not in line with the results of our study, but is consistent with the low chlamydial rectal culture sensitivities noted by Schachteret al.[14] (26.5% to 39.1%) . This disagreement may be attributed to use of rectal sample in such studies **.** As regard the results of cytological examination by giemsa stain in comparison with nested PCR results in diagnosis of genital *C.trachomtis*, out of (34) positive cases of PCR: 17(50 %) patients were positive for cytological examination. And out of (16) patient negative for nested PCR, (0 %) patients was positive for cytological examination. This difference between positive and negative results was considered to be significant. The sensitivity specificity positive predictive value (PPV)and negative predictive value (NPV) of the cytological examination in relation to nested PCR were 50 %, 100%, 100% and 48.5% respectively. ROC curve analysis revealed that cytological examination is a fair test in relation to PCR.Our results are in agreement with Holland and Roberts [15] who perform a comparative study between PCR positive cases for *C. trachomatis* and negative cases as regard abnormal pap smear. They reported a significant difference between positive and negative cases as regard cytological examination**.** In India, Palayekar et al.[16] performed a study on females attending family welfare clinics of Institute for Research in Reproduction. They reported that, cytological examination of endocervical swab for detection of *C. trachomatis* has less sensitivity and good specificity, in relation to PCR , and their results were in agreement of our results**.** On the other hand, Baka et al.[17] who performed a study on asymptomatic non pregnant women of reproductive age presenting to the Obstetrics and Gynaecology Outpatient Clinic of Aretaieio University Hospital, suggested a non significant relation between positive cytological smear and proved cases of a genital *C. trachomatis* infection, and this may be attributed to the method of sampling and transport, as *C. trachomatis* need special transport media**.**

As regard personal history of studied patients in relation to positive cases of *C.trachomatis* as proved by nested PCR, The differences between positive and negative results were insignificant regarding age group, menstrual history, history of abortion, fertility condition and contraceptive method used. Our results are in line with Carlin and Boag[18], Rashidi et al.[19], Eslami et al.[9]. In the contrary, our results disagree with Torrone et al.[20], Forcey et al.[21], Marashi et al.[22], Taylor and Haggerty[23]. These difference could be related to how sampling and methodology were conducted. As regarding presenting symptoms of the studied patients in comparison with positive and negative *C. trachomatis* cases as proved by nested PCR. The differences between positive and negative results were significant regarding abnormal vaginal discharge and cervical erosion and were insignificant regarding post-coital bleeding and associated arthritis. Our results are in accordance with Hajikhani et al.[3], Tosun et al. [24], Yazdi et al. [25], Carter and Hudson[26]. On the other hand, Taylor and Haggerty[23] and Bas et al.[27] disagree with our results. These difference may be attributed to different socio-economic states and hygienic condition, and different patients complaints.

CONCLUSION

From this study we can conclude thattissue culture is a good test in relation to nested PCR, however , cytological examinationis a fair test in relation to nested PCR in diagnosis of genital *C. trachomatis* infection. We recommend the use of nested PCR for detection of genital *C.trachomatis* . Inspite of high cost , it is more specific and sensitive than other traditional methods.

REFERENCES

1. Millman K , Black CM, Johnson RE, Stamm WE, Jones RB, Hook EW. Population-based genetic and evolutionary analysis of Chlamydia trachomatis urogenital strain variation in the United States.J Bacteriol.2004; 186(8):2457–65.
2. Patel AL, Sachdev D, Nagpal P, Chaudhry U, SonkarSC, Mendiratta SL. Prevalence of Chlamydia infection among women visiting a gynaecology outpatient department: evaluation of an inhouse PCR assay for detection of Chlamydia trachomatis. Ann ClinMicrobiol Antimicrob.2010 ; 9:24.
3. Hajikhani B, Motallebi T, Norouzi J, Bahador A, Bagheri R, Asgari S, Chamani-Tabriz L.Classical and Molecular Methods for Evaluation of Chlamydia trachomatis Infection in Women with Tubal Factor Infertility.J ReprodInfertil.2013 ;14(1):29-33.
4. Boyadzhyan B, Yashina T, Yatabe JH, Patnaik M, and Hill CS. Comparison of the APTIMA CT and GC Assays with the APTIMA Combo 2 Assay, the Abbott LCx Assay, and Direct Fluorescent-Antibody and Culture Assays for Detection of Chlamydia trachomatisand Neisseria gonorrhoeae. J. Clin. Microbiol.2004; pp. 3089-3093.
5. Fallah F, Kazemi B, Goudarzi H, Badami N, Doostdar F, Ehteda A, Naseri M, Pourakbari B, and Ghai M.Detection of Chlamydia trachomatiis from urine specimen by PCR in women with cervicitis .Iranian J Publ Health2005 ; 34(2):20-26.
6. Agha SA, El-Mashad N, Rakha SA, and El Metwally A. Value of direct antigen detection methods in diagnosing women with suspected genital Chlamydia trachomatis infection .African Journal of Microbiology Research . 2011; 5(10): 1215-1219.
7. Cheesbrough M. Microscopical techniques used in microbiology. In: Cheesbrough, M. (ed) District Laboratory Practical In Tropical Countries. Part 2. Cambridge University Press 2006; 44-45.
8. Kaye M , Druce J, Tran T, Kostecki R, Chibo D, Morris J, Catton M, and Birch C. SARS–associated Corona virus Replication in CellLines. Emerging Infect. Dis.2006 ;12(1): 129-133.
9. Eslami G, Goudarzi H, Taheripanah R, Taheri S, F Fallah F, Moazzami B, Taherpour A, Ohadi E, Pourkaveh B, Zahirnia Z.Chlamydia trachomatis Detection by Nested-PCR Method on FemalesReferred to Medical Centers of Tehran, Iran .Arch Clin Infect Dis. 2012 ; 7(4): 124-7.
10. Aggarwal SK , Reddy BS, Bhalla P, Kaur H. Utility of direct fluorescent antibody test for detection of Chlamydia trachomatis and its detection in male patients with non-gonococcal urethritis. Indian J DermatolVenerolLeprol .2003 ;69:144-7.
11. Cheng H, Macaluso M , Vermund SH, and, Hook EW.Relative Accuracy of Nucleic Acid Amplification Tests and Culture in Detecting Chlamydia in Asymptomatic Men. Journal of Clinical Microbiology .2001; p. 3927–3937.
12. Johnson RE, Newhall WJ, John R, Papp JR, Knapp JS, Black CM, Thomas L, Gift TL., Steece R , Lauri E, Markowitz LE, Devine OJ, Walsh CM, Wang S, Gunter DC, Kathleen L,Irwin KL, DeLisle S, Berman SM. Screening Tests To Detect Chlamydia trachomatis and Neisseria gonorrhoeae Infections. MMWR Recommendations and Reports 2002 ; 51(RR15);1-27.
13. Bachmann LH, Johnson RE, Cheng H, Markowitz L, Papp JR, Palella FJ, and Hook EW. Nucleic Acid Amplification Tests for Diagnosis of Neisseria gonorrhoeaeand Chlamydia trachomatis Rectal Infections. JOURNAL OF CLINICAL MICROBIOLOGY.2010 ; Vol. 48, No. 5: p. 1827–1832 .
14. Schachter J, Moncada J, Liska S ,Shayevich C , and Klausner JD. Nucleic acid amplification tests in the diagnosis of chlamydial and

gonococcalinfections of the oropharynx and rectum in men who have sex with men.Sex.Transm. Dis. 2008 ;35:637–642.
15. Holland J and Roberts J. Chlamydia trachomatis PCR positivity and inflammatory changes on cervical cytology. Sex TransmInfect .2004 ; 81: 360-361.
16. Palayekar VV, Joshi JV, Hazari KT, Shah RS, and Chitlange SM. Comparison of four non culture tests for Chlamydia trachomatis infection.J Assoc Physicians India.2000 ; 48(5):481-3.
17. Baka S, Tsirmpa I, Chasiakou A, Tsouma I, Politi, E, Gennimata V, and Kouskouni E.Inflammation on the Cervical Papanicolaou Smear: Evidence for Infection in Asymptomatic Women?. Infectious Diseases in Obstetrics and Gynecology .2013 ;Article ID 184302.
18. Carlin EM, and Boag FC. Women, contraception and STDs including HIV.Int J STD AIDS 1995 ;6:373-86.
19. Rashidi BH, Chamani-Tabriz L, Haghollahi F, Jeddi-Tehrani M , Naghizadeh MM , Shariat M. Effects of Chlamydia trachomatis Infection on Fertility; A Case-Control Study. J ReprodInfertil.2013 ;14(2):67-72.
20. Torrone E, Papp J, and Weinstock H.Prevelance of Chlamydia trachomatis genital infection among persons aged 14-39 years , United states 2007-1012.MMWR . 2014 ;63(38): 834-838.
21. Forcey DS, Hocking JS, Tabrizi SN, Bradshaw CS, Chen MY. Chlamydia Detection during the Menstrual Cycle: A Cross-Sectional Study of Women Attending a Sexual Health Service. . PLoS ONE journal 2014 ; 9(1): e85263 .
22. Marashi SMA, Moulana Z, Fooladi AAI, and Karim MM. Comparison of genital chlamydia trachomatis infection incidence between women with infertility and healthy women in Iran using PCR and immunofluresence methods . Jundishapur J Microbiol.2014 ; 7(4): e9450.
23. Taylor BD, and Haggerty CL. Management of Chlamydia trachomatis genital tract infection: screening and treatment challenges. Infect Drug Resist. 2011;4:19–29.
24. Tosun I, Cihanyurdu M, Kaklikkaya N, Topbas M., Aydin F, and Erturk M. Asymptomatic Chlamydia trachomatis infection and predictive criteria among low risk women in a primary case setting . Jpn. J Infec.Dis 2008; 61:216-218.
25. Yazdi JZ, Khorramizadeh MR, Badami N, Kazemi B., Aminharati FZ, Eftekhar Z, Berahme A, Mahmoudi M. Comparative Assessment of Chlamydia trachomatis Infection in Iranian Women with Cervicitis: A Cross-Sectional Study.Iranian J PublHealth 2006 ; Vol. 35 (2) :69-75.
26. Carter JD, and Hudson AP.The evolving story of Chlamydia-induced reactive arthritis. CurrOpinRheumatol.2010; 22:424–30.
27. Bas S, Scieux C, and Vischer TL. Male sex predominance in Chlamydia trachomatis sexually acquired reactive arthritis: are women more protected by anti-chlamydia antibodies?. Ann Rheum Dis 2001 ;60 : 605–611.