University of Thi-Qar Journal Vol.14 No.1 Mar 2019
Web Site: https://jutq.utq.edu.iq/index.php/main
Email: journal@jutq.utq.edu.iq

The frequency of *Chlamydia trachomatis* colonization among infertile women in Al-Nasiriyah city–south of Iraq

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Abstract

Chlamydia trachomatis one of the main causes of pelvic inflammatory diseases which leading to infertility condition especially in women. In this study, 63 vaginal swab specimens from infertile women in Nasiriyah city (south of Iraq) were collected and analyzed by polymerase chain reaction (PCR) by using specific bacterial DNA primer and the positive results correlate in relation to age and types of infertility (primary and secondary). The current study showed that only 9.5% of the samples had the DNA of bacteria. The bacterial infection rate was similar between the primary and secondary types of infertile women. Also, this study found that there were no significant differences between the age of patients and infection and not in the age of infected women between primary and secondary type under ($P > 0.05$). In conclusion, the C. trachomatis may represent an important cause of infertility in samples of Iraqi women with no significant correlation between the infection, age and types of infertility.

Keywords: Infertility, Chlamydia infections, Tubular infertility.

Introduction

Chlamydia trachomatis is an obligate intracellular bacteria that sexually transmitted and can cause a variety of diseases for human such as trachoma, inclusion conjunctivitis, genital tract infections such as: male non-gonococcal urethritis and female cervicitis which may progress to cervical squamous cell carcinoma [(1)] or to serious disorders, such as endometritis, salpingitis and pelvic inflammatory disease, resulting in infertility and ectopic pregnancy and neonatal pneumonia [(2)]. About four million uro-genital infections occur annually in the United States. Many are asymptomatic and can recur, constituting a large reservoir of untreated individuals who can transmit the organism and develop sequelae such as infertility because the infection can damage the fallopian tubes and became a life-threatening ectopic pregnancy [(3)]. *C. trachomatis* also increases the risk for invasive squamous-cell carcinoma of the cervix [(4)] and HIV-1 transmission [(5)]. Identification of *C. trachomatis* genital tract infections has important role in epidemiologic information's about genital tract infections, determination of cure parameters and development of complications [(6, 7)].

Chlamydia trachomatis transmitted through the genital, nasal or rectal mucosa. Infections with *C. trachomatis* are asymptomatic in nearly 60% of cases and the mild symptoms could be seen as vaginal secretion, yellow or green discharge, and diminished cervical mucus during ovulation. While, symptomatic infection is associated with local irritation, mild discomfort to disabling painful intercourse usually linked to cystitis and other signs of urination [(8, 9)].

Infertility is a worldwide problem in male and female associated with a variety of causes including: hereditary, physiologic and infection process. Infertility classified into two types: Primary infertility that refers to couples who have not become pregnant after at least one year having sex without using birth control methods, and Secondary infertility which refers to couples who have been able to get pregnant at least once, but now are unable[(8)].

In female, high rate of secondary infection with *C. trachomatis*, asymptomatic Chlamydia infections like salpingitis is a common cause of tubal damage and tubular infertility which may occur due to the immunological reactions between Chlamydia antigens and antibody[9].

The study designed to detect the *C. trachomatis* infection among infertile women in Thi-qar province/Iraq and their relation to age and types of infertility.

Materials and Methods

Specimens

A total of (63) vaginal swab specimens were collected from infertile women in infertility unit/ Al-Hussein teaching hospital Thi-qar province/Iraq from June to August, 2015 and they divided into (2) groups : first including (42) female with primary infertility and the second including (21) female with secondary infertility.

DNA extraction

The DNA extraction was made according to the genomic DNA purification Kit (Sacace-Italy).

Amplification

The *C. trachomatis* primer was *orf 8* gene was detect using specific primer according to Lee *et al.*, 2007 (f5'-CTAGGCGTTTGTACTCCGTCA) and (r5'-TCCTCAGGAGTTTATGCACT) which amplifies a 200 bp DNA fragment (Bioneer, Korea). The PCR mixture using in amplification containing 4 µl of extracted DNA, 5 µl of mastermix (Bioneer, Korea), 3µl of forward primer, 3 µl of reverse primer and 5µl of DNase free water, the final volume of reaction mixture was 20 µl. Amplification involved one cycle at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 40 sec, annealing at 54°C or 56°C for 45 sec, and extension at 72°C for 80 sec. Finally, the reaction followed by one last cycle at 72°C for 5 min. by using a PCR system (ESCO - Italy) [10].

Statistics

The obtained data were evaluated by Chi square in SPSS program version 14.

Results

The frequency of *C. trachomatis* infections among the infertile women was (6) cases figure (1) which represent (9.5 %) among (63) infertile women involving in this study as showed in table (1).

Table (1): frequency of *C. trachomatis* in infertile women.

C. trachomatis status	Frequency	Percent (%)
Positive	6	9.5
Negative	57	90.5
Total	63	100

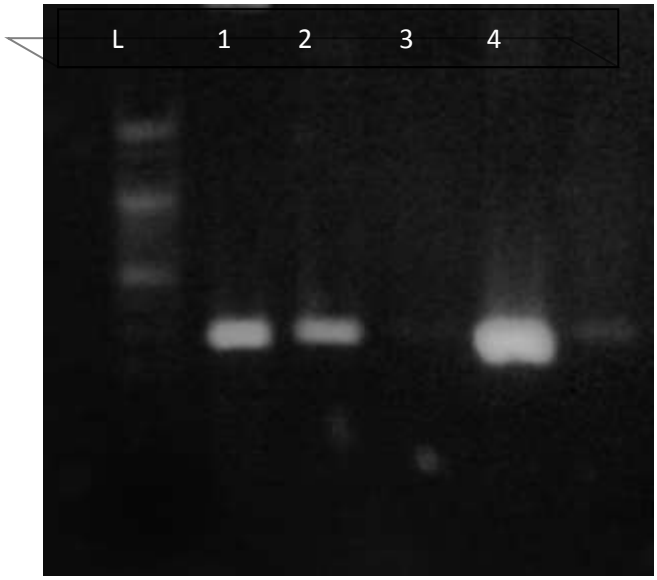

Figure (1): Agarose gel electrophoresis of PCR products of *Orf8* amplicon for *C. trachomatis* that give a PCR products of (200) bp . L: ladder; other lanes represents the DNA from infertile women. Lanes

1,2,4,5 gives PCR products of 200 bp for *C.trachomatis*, lane 3 not shown PCR products.

Table (2) shown no significant differences (P > 0.05) between the ages of infertile women and the infections with *C. trachomatis*. The mean of age of non-infected women was (30.17±9.948 S.D.).While, the mean of age in infected women was (29.05±6.720 S.D.).

Table (2): The relationship between the age and *C. trachomatis* infection.

C. trachomatis status	No.	Mean age	Std. deviation
Infected	6	30.17	9.948
Non infected	57	29.05	6.720

This study showed that the frequency of infections in the primary infertility positive cases was 4 (9.5 %). While, in the women with secondary infertility was 2 cases (9.5 %) with no significant differences among these two groups (P> 0.05) as shown in table (3).

Table (3): The distribution of *C. trachomatis* infections according to the types of infertility.

Types of infertility	*C. trachomatis* status		Total number
	Positive	Negative	
Primary	4 (9.5%)	38 (90.5%)	42
Secondary	2 (9.5%)	19 (90.5%)	21
Total	6 (9.5%)	57 (90.5%)	63 (100%)

Discussion

Chlamydia infections is a more prevalent sexually transmitted disease among young women, re-infection can occur as asymptomatic infection and its lead to development of pelvic inflammatory disease with subsequent complications like infertility. However, as it is commonly

asymptomatic in female, infection may go undetected for potentially lengthy periods of time [11, 12].

Observations in infertile women suggest that an equally large number of women may have post-infectious tubal infertility also after asymptomatic salpingitis; in the majority of cases with serologic evidence of a passed genital Chlamydia infection. Recently, a hypothesis has been presented that those antigen-antibody reactions to Chlamydia heat-shock proteins might be an important factor for the morphological tissue damage and scarring leading to impaired fertility [13].

Our results were consistent with some studies as they showed high incidence of *C. trachomatis* infections among infertile women which may represent an important cause of infertility over the world [14, 15]. Some articles indicate high rates of Chlamydia infections among infertile women in comparison with our study [16, 17]. The differences in infection rates may be due to the differences in socio-economic conditions and the methods that used to detect the incidence of infections in each study. In this study, PCR technique was used to detect previous bacterial infection while the difference of disease outcome of other studies may be due to using of serological tests for diagnosis. Also, the subclinical infections represent a major cause of tubal infertility which difficult to be diagnosed clinically or by some inaccurate laboratory techniques which differ in sensitivity and specificity. Therefore, development of sensitive, specific, and rapid methods to diagnose these infections is highly favored [18].

There are no significant differences in the relationship between the incidence of Chlamydia infections and the types of infertility which may be due to the social conditions and types of screening tests which differ from several studies that indicate high prevalence of Chlamydia infections among women with primary infertility than in secondary one.

Conclusion

The study showed a low percentage of *Chlamydia trachomatis* infection between primary or secondary infertile women that carry Chlamydia DNA within vaginal secretions in equally rates. Furthermore,

there are no significant differences in age of infected and non-infected infertile women.

References

1. Tarja Anttila et al (2001). Serotypes of *Chlamydia trachomatis* and Risk for Development of Cervical Squamous Cell Carcinoma. JAMA, January 3, 2001—Vol 285, No. 1
2. Yong, D.T. and Paul, N.R. (1986). Micro Direct Inoculation Method for the Isolation and Identification of *Chlamydia trachomatis*. J. Clin. Microbiol.: 536-538
3. Centers for Disease Control and Prevention 2005
4. Anttila T., Saikku P., Koskela P., Bloigu A., Dillner J., Ikaheimo I., Jellum E., Lehtinen M., Lenner P. and Hakulinen T. (2001). Serotypes of *Chlamydia trachomatis* and risk for development of cervical squamous cell carcinoma. JAMA. 285:47–51. [PubMed]
5. Lavreys L., Chohan V., Overbaugh J., Hassan W., McClelland R.S., Kreiss J., Mandaliya K., Ndinya-Achola J. and Baeten J.M. (2004). Hormonal contraception and risk of cervical infections among HIV-1-seropositive Kenyan women. AIDS. 18:2179–2184.
6. Byrne, G.I. (2010). *Chlamydia trachomatis* strains and virulence: rethinking links to infection prevalence and disease severity. J Infect. Dis. 15; 201(2): 126–133.
7. Westrom L.V. (1996). Chlamydia and its effect on reproduction. J. Br. Fer. Soc. 1(1):23-30. (Pub Med)
8. Dinah Chelagat (2017).Infertility in Africa: A Great Manifestation of Gender Discrimination. Journal of Humanities and Social Science. Vol. 22, Issue 3, Ver. VII. PP 27-29
9. Brooks G.F., Carroll K.C., Butel J.S. and Morse S.A. (2007). Jawetz, Melnick, & Adelberg's Medical Microbiology. 20th ed. McGraw-Hill Companies.
10. Lee, S.R., Chung, J. M. and Kim, Y.G. (2007). Rapid One Step Detection of Pathogenic Bacteria in Urine with Sexually Transmitted Disease (STD) and Prostatitis Patient by Multiplex PCR Assay (mPCR) . J. Microbiol. 45 (5): 453-459.
11. Sellors, J.W., Mahony, J.B., Chernesky, M.A. and Rath, D.J. (1988). Tubal factor infertility: an association with prior

Chlamydia infection and asymptomatic salpingitis. Fertile Sterile. 49(3):451-7.

12. Millman, K., Black, C.M., Johnson, R.E., Stamm, W.E., Jones, R.B., Hook, E.W., Martin, D.H., Bolan, G., Tavare, S. and Dean, D. (2004). Population-based genetic and evolutionary analysis of *Chlamydia trachomatis* urogenital strain variation in the United States. J Bacteriol. 186:2457–2465.
13. Available from Sheffield Hallam University Research Archive (SHURA) at: http://shura.shu.ac.uk/3371/
14. Cetin MT, Vardar MA, Aridogan N, Köksal F, Kiliç B, Burgut R. (1992). Role of *Chlamydia trachomatis* infections in infertility due to tubal factor. Indian J Med. Res. 95:139–43. [PubMed]
15. Al-Ramahi M, Mahafzah A, Saleh S, Fram K. (2008). Prevalence of Chlamydia trachomatis infection in infertile women at a university hospital in Jordan. East Mediterr Health J. 14(5):1148-54
16. May K. Ismail and Amer S. Ali. (2012). Evaluation of *Chlamydia trachomatis* antibodies in women with infertility. Al- Mustansiriya J. Sci. 23(3): 21-28.
17. de Lima Freitas NS, Borborema-Santos CM, Barroso Serrão das Neves D, Costa de Oliveira CM, Dutra Ferreira JR, Astolfi-Filho S. (2011). High prevalence detection of *Chlamydia trachomatis* by polymerase chain reaction in endocervical samples of infertile women attending university hospital in Manaus-Amazonas, Brazil. Gynecol Obstet Invest.72(4):220-6.
18. Bahareh Hajikhani, Tayebeh Motallebi Jamileh Norouzi, Abbas Bahador, Rezvan Bagheri, Soheila Asgari, andLeili Chamani-Tabriz (2013) Classical and Molecular Methods for Evaluation of *Chlamydia trachomatis* Infection in Women with Tubal Factor Infertility. J Reprod Infertil. 14(1): 29–33.

University of Thi-Qar Journal Vol.14 No.1 Mar 2019
Web Site: **https://jutq.utq.edu.iq/index.php/main**
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تواتر الإصابة بالمتدثرات الحثرية في النساء العقيمات في مدينة الناصرية ـ جنوب العراق

ملخص

المتدثرات الحثرية *Chlamydia trachomatis* واحدة من الأسباب الرئيسية لأمراض التهاب الحوض مما يؤدي إلى حالة العقم وخاصة في النساء. في هذه الدراسة، تم جمع 63 عينة مسحة مهبلية من النساء العقيمات في مدينة الناصرية (جنوب العراق)، تم تحليلها من خلال التفاعل التكراري المتسلسل للحامض النووي البكتيري باستخدام تقنية PCR تفاعل السلسلة المتبلمرة والنتائج الموجبة ربطت إحصائيا بعمر المريضة وأنواع العقم (الابتدائي أو الثانوي). وأظهرت دراستنا أنه في 9.5٪ فقط من حجم العينة كان الحمض النووي البكتيرية موجودا. وكان معدل الإصابة الكلاميدية متماثل بين النوعين الأول والثاني من النساء العقيمات. كما وجد أنه لا توجد فروق ذات تأثير معنوي إحصائي في عمر المريض بين المصابات بالجرثومة وغير المصابات ولا في أعمار المصابات بين النساء العقيمات من النوع الأولي أو الثانوي. بناءا على الدراسة الحالية نستنتج, إن بكتريا الكلاميديا هي سبب مهم للعقم في النساء العراقيات مع عدم وجود علاقة معنوية الإصابة و عمر المريضة و نوع العقم.

الكلمات المفتاحية : العقم، الالتهابات الكلاميدية، العقم أنبوبي.