Pathophysiological Changes in Infertile Women Infected with *Chlamydia Trachomatis*

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Abstract

*Chlamydia trachomatis*is obligatory intracellular gram-negative bacteria that mainly infect the female urethra, cervix and fallopian tubes which is the popular bacterial of sexually transmitted infection (STI) globally. The objective of this study was to determine the infertility of infected women with *C. trachomatis.* This study was conducted at Obstetrics and Gynecology department and fertility center at AL-SADER teaching hospital. Two hundred female were enrolled in current study were divided one hundred of those female was considered the study group with unexplained primary infertility and other100 females were considered as control group. *Chlamydia trachomatis* was detected by ELISA and PCR. Ultrasound examination, Hysterosalpingogram (HSG) for infertile women and Hormone tests included LH, FSH, E_2, Prolactin and Testosterone were achieved all for all women participated in this study during the period from November 2013 to January 2015. The results of this study showed the mean age of the women was ranged from 20 to 45 (29.93 ± 0.41 years). The detection of *C. trachomatis* using PCR showed 17 (17%) infected, while by ELISA was 16 (16%) among control group. However, subfertile group showed 36 (36%) positive detection of Chlamydia infection using PCR, while 33 (33%) infected using ELISA test with a significant positive correlation between Chlamydia infection, fallopian block age and infertility of women. There was non-significant differences in the serum FSH, LH, E_2, testosterone and prolactin levels among all groups. However, the endometrial thickness of subfertile group with Chlamydia infection showed decreasing significantly of endometrial thickness compared with other groups. The results of cervix changes examination showed subfertile subgroup with Chlamydia infection increasing scoring significant compared with other groups. The level of serum iron in the subfertile subgroup with Chlamydia infection showed decreasing significantly compared with other groups. In conclusion, this study showed there was a significant positive correlation between Chlamydia infection and women infertility with a significant positive correlation between Chlamydia infection, fallopian blockage, decrease endometrial thickness, cervix changes and decrease serum Iron level.

Introduction

Chlamydia trachomatis are obligatory intracellular gram-negative bacteria that mainly infect the female urethra, cervix and fallopian tubes. *Chlamydia trachomatis* is the popular bacterial sexually transmitted infection (STI) globally. It is chiefly a female's health care matter because the signs and consequences are further harmful to the reproductive system of female (Manavi, 2006). The sequelae of unobserved and untreated infections like salpingitis and pelvic inflammatory disease result not merely to considerable morbidity but far more notably to infertility (Malik *et al*., 2006). It has been documented that about 100 million novel *Chlamydia trachomatis* infections occurs annually worldwide (Joseph *et al*., 2012). It has also been recognized that chlamydial infection symptoms in women include odd discharge of genitalia, redness, painful and recurrent urination, burning and itching in vaginal area, soreness and swelling of the vulva as well as ache in the pelvis during intercourse. Women infected with Chlamydia could be a symptomatic that might cause severe health sequelae (Fageeh *et al*., 2014). The infection can be passed from mother to the baby and may cause premature rupture of membrane, preterm birth, pneumonia and conjunctivitis of the baby's eyes (Numazaki, 2004). Furthermore, it has been suggested that chlamydial genital infections may be a risk factor that ease the sexual transmission of HIV and human papilloma virus (HPV) induced cervical neoplasia, thus improved means for prevention and control of cases are necessary (Joyee *et al*., 2005). More than 18 serotypes of the organism have been recognized. A number of laboratory methods are used to the diagnosis of *Chlamydia trachomatis*, these consist of cytological tests for detection of intracytoplasmic inclusions, cell culture, enzyme immunoassay (EIA), direct immunofluorescence, DNA hybridization techniques and DNA amplification such as polymerase chain reaction (PCR) (Santos *et al*., 2003). The objective of this study was to determine the infertility of infected women with *C. trachomatis.*

Materials and Method

Study Population

This study was conducted at Obstetrics and Gynecology department at AL-SADER teaching hospital. The current work is case control and it was approved by the Ethics Committee of University of Kufa and informed consent obtained from all

participants. Two hundred females were enrolled the current study, one hundred of those female was considered the study group with unexplained primary infertility. While other 100 females were considered as control that have at least one birth before, who attended the consultant fertility center of the hospital during the period from November 2013 to January 2015. Women were with age ranged from 20 to 45 years and female's partner history was recorded. The patients were divided according to their fertility and their association with *Chlamydia trachomatis* infection into two subgroup first was with positive Chlamydia while the second group with negative Chlamydia. All females were examined clinically by gynecologist. The cervical changes were described as normal appearance, inflamed with discharge, ulcerated appearance and lesions on cervix scoring 0,1,2,3 respectively**.**

Hormonal analysis on base line reading of FSH, LH, prostaglandin E_2, prolactin and E_2 were achieved in an early follicular phase (cycle day 2-5). Ultrasound examination of vaginal routinely performed for females attend to fertility clinic at the hospital those whom underlying abnormality such as uterine fibroid, ovarian pathology. Hysterosalpingography (HSG) and/or laparoscopy for assessment of tubal patency is done in a radiological unit for the patient who suspected to have tubal factor(blockage unilateral or bilateral).*Chlamydia trachomatis* was detected by enzyme linked immunosorbent assay (ELISA) then confirmed by polymerase chain reaction (PCR) detection. Serum blood was collected at the morning hours under sterile conditions and stored at -8^0C till the tests were processed for PCR and ELISA analyzed.

Statistical Analysis

Data were collected and analyzed by using Statistical Package for Social Studies (SPSS 19) for descriptive statistics involving means and standard error of mean (SEM). Analysis of variance (ANOVA) was used for more than two groups. Chi square test, Fisher's exact test and odd ratio were done when appropriate.

Results

The mean age of the women ranged from 20 to 45 (29.93 ± 0.41 years), and there was no statistical significance among fertile and subfertile women at (P-value > 0.05). The detection of *Chlamydia trachomatis* using PCR showed the infection amongst control group were 17 (17%) women while by ELISA was 16 (16%). However, the subfertile

group (study group) showed 36 (36%) women positive detection Chlamydia infection using PCR, while 33 (33%) infected using ELISA test, there was significant difference of odd ratio (2.746 at 95% confidence interval 1.416 to 5.328), relative risk and the correlation between infertility rate and Chlamydia infection with pregnancy at P-value (0.004). The results of PCR test was considered more sensitive (Table1).

Table1.Risk estimation, odd ratio with P-value between pregnancy and presence of Chlamydia trachomatis by PCR

Data analyzed	Control	Study
Negative	83 (83%)	64 (64%)
Positive	17 (17%)	36 (36%)
Total	100 (100%)	100 (100%)
P-value	0.004*	
Odds ratio	2.746	
95% confidence interval	1.416 to 5.328	

*P<0.05: Significant difference between negative and positive presence of Chlamydia trachomatis.

This study showed a significant positive correlation between Chlamydia infection and infertility women, the infertility duration for subfertile women with and without infection was 2.89 ± 0.16 and 4.16 ± 0.34 respectively with a significant difference P<0.05 (Table 2).There was 23 (23%) women with fallopian blockage, majority (14) of them were associated with Chlamydia infection with a significant difference between groups at (P<0.05) (Table 2).

Table 2.Comparison of mean infertility duration per year among subfertile groups and fallopian blockage

Group [a]	Subgroups	Mean duration/SE	Minimum	Maximum	Fallopian blockage Yes	No
Subfertile	-ve[a] Chlamydia (n= 64)	2.89 ± 0.16	1	8	9	64
	+ve[a] Chlamydia (n=36)	4.16 ± 0.34	1	8	14	36
Total		3.52 ± 0.10	1	8	23	100

a significantly different with same group.

The overall level of FSH among all groups was 5.20+0.09, in the control group free from Chlamydia infection the mean level of FSH was 5.20 +0.16, while for those with Chlamydia infection was slightly higher 5.29+0.35. On the other hand, the levels of FSH in the subfertile group showed with Chlamydia infection was 5.09+0.16and higher then with without infection 5.33+0.22 (Table 3). The overall level of LH among all groups was 3.84+0.08. The control group without Chlamydia infection showed LH was 3.77+0.15 while for those with Chlamydia infection showed 3.75+0.19. The level of LH for subfertile group showed the higher than with Chlamydia infection4.22+0.21, while the lowest (3.67+0.15) was shown in the group without Chlamydia infection (Table 3).

Table3: The Mean of FSH and LH levels among groups

Group	Subgroups	Mean level of FSH/SE	Mean level of LH/SE
Control	-ve Chlamydia(n=83)	5.20+0.16	3.77+0.15
	+ve Chlamydia (n=17)	5.29+0.35	3.75+0.19
Subfertile	-ve Chlamydia(n=64)	5.09+0.16	4.22+0.21
	+ve Chlamydia (n=36)	5.33+0.22	3.67+0.15
Overall		5.20+0.09	3.84+0.08

[a] There was no a significant difference between groups P value <0.05.

The overall level of E_2 in all groups was 57.73+0.31. The E_2 level of control subgroup without Chlamydia infection was 58.81+0.59, whereas for those with Chlamydia infection was 57.85+0.70. In contrast, E_2 level for subfertile subgroups with and without infection were 58.06+0.64 and 56.89+0.58 respectively, and there was no significant differences among the groups. The overall level of Testosterone amongst groups was 0.522+0.004. The control group without Chlamydia infection showed the testosterone was 0.515+0.007, while those with Chlamydia infection was 0.518+0.009. The levels of testosterone slightly higher in the subfertile subgroups with and without infection were 0.513+0.008 and 0.538+0.007 respectively, and there was no significant differences among the groups (Table 4).

Table 4. Mean of E_2 and Testosterone hormones levels among groups

Group[a]	Subgroups	Mean level of E_2/ SE	Mean level of Testosterone/ SE
Control	-ve Chlamydia (n=83)	58.81+0.59	0.515+0.007
	+ve Chlamydia (n=17)	57.85+0.70	0.518+0.009
Subfertile	-ve Chlamydia(n=64)	58.06+0.64	0.513+0.008
	+ve Chlamydia (n=36)	56.89+0.58	0.538+0.007
Total		57.73+0.31	0.522+0.004

[a]There was no significant difference of E_2 and Testosterone hormones levels among groups

The overall level of prolactin for all groups was 11.86+0.17. The level of prolactin in the control without Chlamydia infection was 11.45+0.32, while those with Chlamydia infection was 11.36+0.34. The levels of prolactin showed higher in the subfertile subgroups with and without infection were 12.31+0.38 and 12.30+0.31 respectively, but there was no significant difference among all groups in hormones (Table 5).

The overall level of endometrial thickness (ET) amongst groups was 8.19 +0.11. The control group without Chlamydia infection showed 8.44+0.13 thickness of endometrial, whereas those with Chlamydia infection showed 8.39+0.2.The levels of endometrial thickness in the subfertile subgroups with and without infection were 9.51+0.21 and 6.7+0.15 respectively. Endometrial thickness of subfertile with Chlamydia infection subgroup showed decreasing of ET significantly compared to other individually (Table 5).

The results of cervix changes examination showed the mean score of the entire women enrolled in the current study was 1.135+0.08. The mean cervix changes of women without Chlamydia infection in control subgroup was 0.22+0.05. While those with infection showed 1.95+0.12. On the other hand, mean scoring of cervix changes in the subfertile women subgroup without Chlamydia infection was 0.37+0.07, while the subfertile subgroup with Chlamydia infection was 2.12+0.14, which this increasing showed a significant differences when compared with other groups (Table 6). The level of serum iron for the total women enrolled in the current study was 87.03+2.40. The iron in the women without Chlamydia infection in control subgroup was 98.40+4.20, while those with infection 68.51+4.44. However, the subfertile subgroup without infection was 103.28+4.30, while subfertile subgroup with infection

showed 75.34±4.41 with decreasing significantly differences when compared with other groups (Table 6).

Table 5.Mean of Prolactin hormone and ET levels among groups

Group	Subgroups	Mean level of Prolactin [a]/ SE	Mean level of ET[b]/SE
Control	-ve Chlamydia(n=83)	11.45±0.32	8.44±0.13
	+ve Chlamydia (n=17)	11.36±0.34	8.39±0.26
Subfertile	-ve Chlamydia(n=64)	12.31±0.38	9.51±0.21
	+ve Chlamydia (n=36)	12.30±0.31	6.7±0.15
Total		11.86±0.17	8.19±0.11

[a]There was no significant difference among all groups, [b]subfertile with Chlamydia infection subgroup was decresing of ET significantly compared to other individually

Table 6.Mean Score of cervix changes and mean level of Iron among groups

Group	Subgroups	Mean score of Cervix changes/SE	Mean level of iron/ SE
Control	-ve Chlamydia (n=83)	0.22±0.05	98.40±4.20
	+ve Chlamydia (n=17)	1.95±0.12	68.51±4.44
Subfertile	-ve Chlamydia (n=64)	0.37±0.07	103.28±4.30
	+ve Chlamydia (n=36)	2.12± 0.14[a]	75.34± 4.41[b]
Total		1.135±0.08	87.03±2.40

[a]Increasing of cervix changes significant differences, [b]decreasing of serum iron significantly differences when compared with other groups

Discussion

The data of the present study showed that the mean of infertility duration was 3.52±0.10 years. The present study showed that the *C. trachomatis* genital tract infection was 17% in control subjects and 36% in infertile women. The detection of *C. trachomatis* infection was found higher in infertile women that may be one of the reason for unexplained infertility and it was mainly to clarify the relationship between *C. trachomatis* of genital infection and its impact on fertility. These results are in agreement with many literatures focusing on infertility duration of women seeking gynecologic treatment for problems of infertility. These results are approximately similar to the finding of the present study and the minor differences are certainly due to geographic and sample size differences (Dhananjaya *et al.,* 2014) peak duration as

4-6 years mean duration 4.62+1.58. Taha and Khanzad (2013) found the mean duration was 7.53+5.69. Bayan and Shahla (2013) found that >2 years. African countries are developing countries in which the prevalence of *C. trachomatis* infection and its relation to female infertility is of prime importance to be compared with the results of the present study as it shares the criteria of the environmental effect of developing countries was estimated the significance of *C. trachomatis* in infertile women (Jorn *et al.,* 2008). The this African study enrolling 439 women as 191 with primary or secondary infertility the odd ratio was 2.1-2.8 and IgG was 39% verses19% *C. trachomatis* effect on fertility rate, a fact which is similar the results of the present study. In an Indian study, the rate of *C. Trachomatis* infection in relation to infertility was estimated that *C. Trachomatis* infection was found in 13.5% (27/200) infertile women by in-house real time-PCR (Mohammed *et al*., 2012), 11.5% (23/200) (Benu Dhawan *et al*., 2014). Although, the rate of *C. Trachomatis* infection is less than that obtained in the present study, it still emphasizes the strong relation between *C. Trachomatis* infection and infertility in Iraqi to estimate the relation between *C. trachomatis* infection and infertility (May and Amer 2012). The results of this study showed that the mean age of infertile women was 30±4.12, which is similar to the result of the present study, and that the ranged from 20 - 40 years. The result of previous study showed that 25 % had IgG serological evidence for *C. trachomatis* infection which is approximately similar to the result of the present study. In another on 110 infertile women, the following results showed the *C. trachomatis* was detected in 31 (28.1%) of thee110 infertile women, while one (3.3%) in control group was positive for C. trachomatis (P<0.01) (Malik *et. al.* 2006). This result is in accordance with the results of the previous study which stated thate38% women with Chlamydial infection also had tubal occlusion, making a highlight on the strong relationship between infection and tubal blockage as a cause of infertility. Again this result is in accordance with the previous study where a clear was no effect of *C. trachomatis* on hormone change. This study show low endometrial thickness to the female have *C. trachomatic* infection so maybe effect on endometrial thickness compared with female without infection. The present study demonstrated that the women have *C. trachomatis* infection have low iron level of infertile women compared to the other group, and low endomaterial thickness compared to other group. *Chlamydia trachomatis* has impact on fallopian tube that may be possibly cause blockage to fallopian tube if not treated early Chlamydia effects on cervix which may lead to

discharge, ulceration and cervicitis. In conclusion, this study showed there was a significant positive correlation between Chlamydia infection and women infertility with a significant positive correlation between Chlamydia infection, fallopian blockage, decrease endometrial thickness, cervix changes and decrease serum Iron level.

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