IMPACT OF GENITAL CHLAMYDIA TRACHOMATIS INFECTION IN WOMEN PRESENTING WITH INFERTILITY IN LAGOS, NIGERIA.

ABSTRACT

Background: Chlamydia trachomatis is an obligate intracellular bacterial pathogen that has been implicated in a wide spectrum of human reproductive diseases including infertility. Many studies have confirmed the association of Chlamydia trachomatis with tubal infertility. However, there is a paucity of information on its contribution to various other types of infertility including unexplained infertility. The available studies on the association of chlamydia with infertility are serology based.

Objectives: Using nucleic acid amplification test (NAAT), this study determined the association between Chlamydia trachomatis infection and infertility in women attending the infertility clinic of the Lagos University Teaching Hospital.

Methods: This was an analytical cross- sectional study conducted among reproductive age women with infertility and an equal number of fertile women attending the Lagos University Teaching Hospital. Chlamydia trachomatis detection test (NAAT) was done using laboratory protocol comprising nucleic acid amplification test.Data analysis was performed using the EPI-INFO version 7.2 statistical software, with appropriate statistics and associations carried out.

Results: The prevalence of chlamydia trachomatis infection was 10% in infertile participants but 1.7% in the fertile comparative group (P=0.057). There were statistically significant differences in age ($p = 0.016$) and parity ($p = <0.001$) of women in the two comparison groups. Chlamydia trachomatis infection positivity was higher in women in the third decade of life on multivariate analysis($p = 0.008$). Half of the women with infertility that tested positive for chlamydia trachomatis infection in this study had tubal factor while the other half had unexplained infertility.

Conclusion: This study showed a higher prevalence of Chlamydia trachomatis infection in women with infertility as compared with women with spontaneous intrauterine pregnancy. There was no association between active Chlamydia trachomatis infection and infertility.

Key Words

Chlamydia trachomatis, infertility, nucleic acid amplification, Nigeria.

INTRODUCTION

Infertility is a disease of the reproductive system defined by the failure to achieve pregnancy after 1 year or more of regular unprotected sexual intercourse.[1] This is said to be primary infertility when a woman has never been able to achieve pregnancy and secondary infertility when a woman that has previously been pregnant is unable to achieve pregnancy.[1] About 1 in 6 of women of reproductive age will present with one form of infertility or the other.[2]Infertility has several consequences which impacts negatively on every aspect of couples life ranging from psycho-social, economic, religious, and clinical.[2, 3, 4]Infertility in Nigeria is majorly secondary and infection has been a prominent causal factor that usually presents as chronic pelvic inflammatory disease and tubo- ovarian abscess with sequelae of functional and structural damage of the fallopian tube. [2. 5, 6- 9]

Chlamydia trachomatis is an obligate intracellular bacterial pathogen and it is a major cause of genital tract infection and sexually transmitted infection with its ability to evoke both cellular and humoral immune responses leading to activation of macrophages, polymorphonuclear leucocytes, and release of cytokines.[10-13] These immunological factors influence various aspects of reproduction including follicular development, oocyte quality, ovulation, luteinization, fertilization, implantation, fetal development and pregnancy immune tolerance.[14, 15] Also, Chlamydia trachomatis induces increased production of reactive oxygen species and nitric oxide leading to oxidative stress which can cause suboptimal tubal function, and tubal dysfunction; thus affecting adequate ciliary motion and muscular activity necessary for sperm–oocyte interaction and transport of the embryo to the uterine cavity for implantation.[10- 14]

Chlamydia trachomatis is the commonest pathogen implicated in most cases of infertility.[2, 7]Chlamydia trachomatis infects the epithelial cells and fibroblast with a predilection for mucous

membranes. Replication of this bacterium in infected host cells varies considerably and may be low, especially in asymptomatic and persistent infections. Thus, its detection requires a test with high sensitivity and specificity.[10, 16-18] The gold standard of diagnosis of chlamydia trachomatis is Nucleic Acid Amplification Test (NAAT) which detects the bacterial deoxyribonucleic acid (DNA), it is the most sensitive (95-98%) with specificity (more than 99%) comparable with that of cell culture (sensitivity 40-70%, specificity more than 99%). NAAT for chlamydia is effective for large-scale screening with rapid turn-over time and it has been approved on all specimen types including the non-invasive urine specimen. However, most of the studies done in this environment are serology-based studies which are less sensitive (60-70%) and less specific (95-99%) for the pathogen.[18-25.]

Many studies have also confirmed the association of Chlamydia trachomatis with tubal infertility. However, there is a paucity of information impact of Chlamydia infection on various other types of infertility including unexplained infertility. This study, therefore, aimed to investigate the magnitude of the contribution of Chlamydia infection to infertility by determining the association between chlamydia trachomatis infection and infertility using the more accurate nucleic acid amplification test for chlamydia detection on self-collected urine and vaginal samples.

MATERIALS AND METHODS

Study design and site: This was an analytical cross- sectional study carried out at the gynaecological and antenatal clinics of the Lagos University Teaching Hospital (LUTH), Lagos, South-west Nigeria between May 1st and September 30th, 2021.

Participants' selection:

Eligible participants in the case group were women diagnosed with infertility using clinical history, gynecological examination and basic investigations: pelvic ultrasound, hormone profile and hysterosalpingogram. While the comparison group included age-matched (+/- 2years) pregnant women at less than 24 weeks gestational age with no history of or prior treatment for infertility.

Excluded from the study were women with active or recently treated pelvic inflammatory disease in the past 1-month, previous history of ectopic pregnancy, antibiotics use in the past 4 weeks and with established male factor infertility

Sample Size Determination:

The sample size was determined by applying the formula for comparison of two proportion[26].

$$n = \frac{(u + V)^2\{p_1(100 - p_1) + p_2(100 - p_2)\}}{(p_1 - p_2)^2}$$

$n =$ minimum sample size for each group

u = one-sided percentage point of the normal distribution, corresponding to 1- the power.

v = Percentage point of the normal distribution, corresponding to the (two-sided) significance level.

p_1 = the estimated percentage of an attribute that is present in population 1 (patients with infertility)

p_2 = the estimated percentage of an attribute that is present in population 2 (fertile patients)

At 95% confidence level, with 80% power, v = 1.96 and u = 0.84

A previous Nigeria study[27] found that the prevalence of chlamydia infection among women with tubal infertility was 61.7%.

While prevalence of chlamydia infection in women with normal fertility was 34.0%

Then, p_1= 61.7% and p2 =34.0 %

$$n = \frac{(0.84 + 1.96)^2\{61.7(100 - 61.7) + 34(100 - 34)\}}{(61.7 - 34)^2}$$

n =47.07

Hence, the calculated minimum sample size for each group was 47.07

Assuming an attrition rate of 20% then the minimum sample size was 47.07+9.414=56.484 =57

The minimum sample size for each group was 57 and this was approximated to 60.

The total sample size was 120.

Participants Recruitment

Potentially eligible participants were counselled individually during which the purpose of the study was explained and written informed consent was obtained. A proforma was used to obtain information on the socio-demographic characteristics and the pattern of sexual behaviour of the study participants. Relevant information obtained were -participant's age, parity, level of education, occupation, occupational status of the husband, marital status, previous use of Intra-Uterine Contraceptive Device (IUCD), age at sexual debut, number of sexual partners and number of husband's sexual partners.

Study procedures

Following participants' recruitment, self- sampled urine and vaginal swab were collected, and study numbers were assigned to each participant to ensure anonymity.

At least 20ml of the first portion of a void urine self- sample were collected into a sterile universal bottle with the appropriate study number was provided by each participating woman. No vulva washing with or without antiseptic solution was necessary prior to the urine collection.

Vaginal swab self–samples were also collected with a swab stick with the appropriate study number on the swab reagent tube. At least 5cm (2 inches) of the swab stick was inserted into the vagina and rotated the swab at 360°for 10-30seconds. No vulva washing was necessary prior to the vaginal swab collection.

LABORATORY METHODS

Principle of Test

The nucleic acid amplification test detects the DNA material of Chlamydia trachomatis. It was performed on vaginal swab and urine samples thus eliminating invasive techniques and the need to invade participants' privacy while passing speculum. The kit used in the DNA isolation was Norgens's Urine DNA isolation kit for exfoliated cells or bacteria, which was based on the principle of spin column chromatography. The genomic DNA was preferentially purified from other cellular components such as proteins and RNA: This was done by suspension and vortex, then lysing with Protein Kinase K and lysozyme. The mixture was loaded into a spin column that binds DNA in a manner that depends on ionic concentrations, thus only DNA would bind to the column while most of the RNA and proteins would be removed in the flow-through. The bound DNA was double washed to remove any remaining impurities and then eluted with a buffer. Polymerase chain reaction (PCR) was carried out to amplify the Chlamydial trachomatis gene. A second PCR was carried out to confirm that the isolation of bacteria DNA was properly done, followed by gel electrophoresis to identify the amplified gene

PROCEDURE

The laboratory analysis was done in LUTH molecular laboratory. The procedure was piloted using 5 samples, then on the rest of the samples. All solutions were brought to room temperature. The urine samples were brought from storage at -80^0C and thawed to room temperature. A working concentration of the wash solution A was prepared by adding 42ml of 100% ethanol to the supplied bottle containing concentrated wash solution A. A stock solution of lysozyme 400mg/ml (1.7x107 units/ml) was prepared aiming at lysozyme activity $\geq$ 20,000 units/mg of protein. The heat block was set at 55^0C.

The DNA isolation was carried out by transferring 10ml of urine into a 15ml centrifuge tube and centrifuged at 3000rpm for 5minutes to pellet the cells. The supernatant was then discarded carefully so as not to disturb the cells. 200ul of resuspension solution A was added to the cell pellets and re-suspended by gentle vortexing. 600ul of lysis buffer B, 12ul of lysozyme stock solution and 10ul of Proteinase K was added to the cell suspension. The mixture was vortexed and incubated at 55°C for 30minutes. 160ul of 100% of Isopropanol was added to the lysate and mixed well by gentle vortexing until a homogenous mixture was obtained. 500ul of the lysate mixture was transferred into the spin column, capped tightly and centrifuged for 2 minutes at 8000rpm. Columns in which the lysate did not completely flow-through were spun for an additional 1minute at 14000rpm. The flow-through was discarded and the centrifugation was repeated for the remaining lysate. 500ul of wash solution A was added to the column and centrifuged for 1minute at 14000rpm. The flow-through was discarded and 500ul of the wash solution A was added again to the column and centrifuged for 1minute at 8000rpm. The flow-through was discarded and the column was spun for 2minutes at 14000rpm.

The spin-column was assembled with the 1.7ml elution tubes and 30ul of elution buffer B was dispensed in aliquot into the columns and centrifuged for 2 minutes at 8000rpm. They were then centrifuged for an additional minute at 14000rpm to collect the total elution volume.

The amplification was done using real-time PCR with SYBR Green as the fluorescent probe. Luna (R)Luna® Universal qPCR Master Mix Protocol (M3003) was followed using 10 µl of the master mix (Luna® Universal qPCR Master Mix),0.5 µl each of forward and reverse primers, 2 µl of template DNA (bacterial DNA) and 7 µl of nuclease-free water to make a 20 µl reaction.

The conditions were 95^0C for 60seconds, 95^0C for 15 seconds, 45cycles of extension (+plate read) at 60^0C for 30seconds and a melt curve step at 60-95^0C with 0.5^0C increment

The gel images were read as well as the PCR results. Samples 42, 46,47,49,52,84 and 134 from both the urine and vaginal swab samples were positive for C. trachomatis.

Laboratory quality assurance and control were ensured by repeating amplification for the pilot DNA using primers designed to amplify the 16s forward and reverse gene sequence which is conserved among bacteria. The DNA yield was confirmed to contain bacteria genome using a primer with optimal annealing temperature 49.5-54°C band 1500 base pairs master mix (NEB SOLAS BIODYNE).

Case definition: Chlamydia trachomatis positivity was defined in this study as women with combined self-sampled urine and vaginal Chlamydia trachomatis infection positivity using NAAT results.

Data processing and statistical analysis

Data were analyzed using computer software packages Epi info version 7 of the Centre for Disease Control (USA, March 2015). Quantitative data were expressed in descriptive statistics (mean ± standard deviation or median± range). Continuous variables were compared using the student's t-test and Mann-Whitney U test as appropriate. Categorical variables were compared using the Pearson's Chi-square test or Fischer test as appropriate. Bivariate and multivariate analyses were done using a binary logistic regression model to control for all the potential confounders of infertility. P-values less than 0.05 was considered to be statistically significant.

Ethical Considerations: This study was carried out after obtaining approval from the Health Research Ethics Committee of the Lagos University Teaching Hospital with health research committee assigned number: **ADM/DCST/HREC/APP/4002.**

RESULTS

Overall, 120 women were recruited for the study, (60 in each group). The prevalence of Chlamydia trachomatis infection was 10% in infertile participants but 1.7% in the fertile comparison group. There was no statistically significant difference in the prevalence of chlamydia trachomatis infection between participants in the infertile and fertile groups (P=0.057).

Chlamydia trachomatis infection positivity was higher in women in the third decade of life (p = 0.008).

Half of the women with infertility that tested positive for chlamydia trachomatis infection in this study had tubal factor (3) while the other half had unexplained infertility (3). However, no significant association with chlamydia trachomatis positivity and clinical diagnosis. (p = 0.776).

DISCUSSION

The prevalence of chlamydia trachomatis infection using the highly sensitive and specific nucleic acid amplification test was 10% in women with infertility and 1.7% in the fertile comparators. Several studies have documented prevalence ranging from as low as 2% to as much as 26% among women of reproductive age.[27-32]. The prevalence is quite high among women with

infertility especially those with tubal factor infertility.[33-41.] The chlamydia trachomatis prevalence of 10% recorded in women with infertility was slightly higher than the 7.3% reported by Ajani et al using the polymerase chain reaction.[42] However, our reported prevalence rate of 10% in infertilewomen population was almost comparable to the prevalence of 13.8% recorded by Hossein in Iran where chlamydia detection was done using polymerase chain reaction. However, a far less prevalence of 8.6% and 0.9% were seen using IgG and IgM in women with infertility as compared to 4.9% and 1.8% using IgG and IgM respectively in the fertile controls. This Iranian study was a pointer to the likelihood of low prior infection perhaps due to the dominating influence of religion and culture on sexual practices and preferences in the country.[43] Many studies in our environment are serology-based thus giving higher prevalence rates of 20% to 65.8% in women with infertility as compared to 17.3% in the fertile control. [27,44-55.]

The rate was also higher in a study by Odusote et al who reported a prevalence of 38.6% in the infertile population and 22.8% in the fertile control,[48] just as Koladade et al reported 31% prevalence in the infertile population and 29.5% in the fertile controls.[49] Prevalence of 38.3% was reported by Tukur et al in women with infertility and 13.3% prevalence in the fertile controls.[50]Sattari et al reported a prevalence of 35.88% in women with infertility and 18% in fertile control.[51] Higher prevalence was reported by Ogbu et al which is 75% prevalence in women with infertility and 23.5% in fertile control.[52] These aforementioned studies showed a higher prevalence of Chlamydia trachomatis infection using serological studies which are indications of previous exposure to the infection unlike the nucleic acid amplification test used in this study which is a test of ongoing infection.

Though the prevalence of chlamydia trachomatis infection was 10% in women with infertility and this is higher than the 1.7% prevalence in pregnant comparison group in this study using

nucleic acid amplification testing but this difference was not statistically significant (p-value =0.057). This is similar to the findings by Olamijulo et al in Lagos university teaching hospital using a serological testing kit,[55] and the findings by Tayo et al in Lagos state university teaching hospital.Though the patients with positive chlamydia assay were 1.747 times at risk of infertility compared to patients with negative chlamydia assay, but this not statistically significant .[46] The finding in this study is also comparable to the findings of Omoh-Agoja et al who reported a statistically higher prevalence (65.8% as compared to 17.3% controls) using serological chlamydia antibody testing which became insignificant when effects of gynecological symptoms, sociodemographic, contraception and sexual history were controlled for in a conditional logistic regression model. Thus, it was concluded that no strong independent association exists between chlamydia antibodies and the risk of being infertile in Nigerian women.[47] In a similar fashion, Hossein et al in Iran using polymerase chain reaction demonstrated a chlamydia trachomatis prevalence of 13.8% in the infertile study group and 11.1% in the pregnant controls with no statistically significant difference .[43]

Ojule et al demonstrated a significantly higher prevalence of Chlamydia trachomatis in women with tubal factor infertility (61.7%) than their pregnant controls (34%).[27] Also Odusote et al found a higher but insignificant chlamydia IgG antibody prevalence (38.6%) in infertile women compared to their pregnant controls (22.8%).[48] This study revealed there was no association between infertility and education status (p=0.72); unlike Koledade et al who reported a high seroprevalence of IgG and IgM to chlamydia trachomatis infection of 31% and 29.5% respectively with a statistically significant association between education status and seropositivity with primary education status having most and tertiary education having least.[49]Ogbu et al demonstrated a statistically significant association between tubal infertility and

chlamydia infection using a serological assay with the presence of chlamydia in 75% of infertile and 23.5% of pregnant control with p-value < 0.001.[52] Nada et al reported 15% detection of chlamydia using a cervical swab of women with unexplained infertility in Egypt as compared to 2% of fertile control with p -value of 0.002.[54] However, all these studies were serology based and only detects prior infection and less accurate as compared to nuclei acid amplification used in this study which is a test of ongoing infection.

This study used both self-collected urine samples and vaginal swabs for nucleic acid amplification test which is the gold standard of diagnosis of chlamydial without undue invasion of participant's privacy. Thus, high detection of ongoing infection is guaranteed without the positivity of prior infections thus reducing detection errors. This will however open the window for further studies to compare Chlamydia trachomatis detection using nucleic acid amplification in cervical secretion in addition to future research on the determination of genomics of the bacteria DNA found in urine and vaginal swabs of the patients studied. This study also showed a **higher C. trachomatis positivity in women aged 20–29. This is similar to report in other studies. Other authors such as** Meneses-León and colleagues also reported similar trend. Increased sexual activity at that age group has been suggested as the most likely cause of this trend.[56]

This study revealed no association between active Chlamydia trachomatis infection and infertility. This study has a few limitations. Firstly, it is hospital-based study and thus may not represent the situation in the general population. Secondly, due to its cross-sectional design, this may not allow us to attribute any causal relationship between Chlamydia trachomatis infection and infertility. However, to the best of our knowledge, this was the first research in Nigeria that used the molecular detection to test association of chlamydia trachomatis and infertility. In addition, the study would provide the much-needed preliminary data for further testing in future

studies in an attempt to make paradigm shift in the prevention of infertility and its management in Nigeria and sub-Saharan Africa.

CONCLUSION

This study showed a higher but statistically insignificant prevalence of Chlamydia trachomatis infection in women with infertility as compared with women with fertile women with spontaneous intrauterine pregnancy. Women with infertility who tested positive for chlamydia trachomatis infection were limited to tubal factor and unexplained infertility in this study.

RECOMMENDATION

The findings of this study may suggest nobasis for the routine screening for chlamydia trachomatis infection in patients with infertility and thus the empiric treatment of such patients may be a waste of resources. However, young adults should be counseled to practice safe sex and prevent unwanted pregnancy as a way to reduce their risk of chlamydia trachomatis infection and subsequent tubal factors and unexplained infertility. Meanwhile, the combined use of nucleic acid amplification test and genome sequencing for ongoing infection and serum antibody for prior infection through robust multi-centre and longitudinal studies will further validate the findings of this study.

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TABLE 1: SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Variables	Cases n= 60(%)	Control n= 60(%)	Total n= 120(%)	P-value
Age (years)				0.016*
20-29	5(8.3)	17(28.3)	22(18.3)	
30-39	45(75.0)	37(61.7)	82(68.3)	
40-49	10(16.7)	6(10.0)	16(13.3)	
Parity-Nulliparous	52(86.7)	24(40.0)	76(63.3)	<0.001*
Primiparous	5(8.3)	13(21.7)	18(15.0)	
Multiparous	2(3.3)	9(15.0)	11(9.2)	
Grand multiparous	1(1.7)	14(23.3)	15(12.5)	
Level of Education- None	0(0.0)	1(1.7)	1(0.8)	0.720
Primary	3(5.0)	1(1.7)	4(3.3)	
Secondary	14(23.3)	13(21.7)	27(22.5)	
Tertiary	43(71.7)	45(75.0)	88(73.3)	
Occupation- Skilled	13(21.7)	21(35.0)	34(28.3)	0.105
Unskilled	47(78.3)	39(65.0)	86(71.7)	
Occupation of Partner- Skilled	24(40.0)	20(33.3)	44(36.7)	0.449
Unskilled	36(60.0)	40(66.7)	76(63.3)	
Previous use of IUCD-				1.000
yes	1(1.7)	0(0.0)	1(0.8)	
no	59(98.3)	60(100.0)	119(99.2)	
Number of sexual partners				0.783
<3	53(88.3)	52(86.7)	105(87.5)	
≥ 3	7(11.7)	8(13.3)	15(12.5)	
Number of husband sexual partners <3	46(76.7)	34(56.7)	80(66.7)	0.020*

>= 3	14(23.3)	26(43.3)	40(33.3)

Table 1: The table revealed that there was significant difference between age (0.016), parity (p value <0.001) and number of husband's sexual partners (p value = 0.020) of the two comparing groups.

TABLE 2: THE MULTIVARIATE ANALYSIS OF THE ASSOCIATION BETWEEN PARTICIPANTS CHARACTERISTICS AND CHLAMYDIA TRACHOMATIS INFECTION.

Variables	OR	P-value	95% CI
Age (years)			
20-29	37.700	0.008*	2.605-545.596
30-39	8.415	0.097	0.679- 104.250
40-49	Reference		
Parity-Nulliparous	0.000	0.998	0.000
Primiparous	0.000	0.988	0.000
Multiparous	0.000	0.988	0.000
Grand multiparous	Reference		
Level of Education- None	0.213	1.000	0.000
Primary	0.277	1.000	0.000
Secondary	0.605	1.000	0.000
Tertiary	Reference		
Occupation- Skilled	2.852	0.074	0.903- 9.006
Unskilled	Reference		
Occupation of Partner- Skilled	0.627	0.411	0.206-1.909
Unskilled	Reference		
Previous use of IUCD-			
yes	0.000	0.999	0.000
	Reference		
no			

Number of sexual partners			
<3	0.979	0.978	0.207-4.631
≥ 3	Reference		
Number of husband sexual partners <3	0.754	0.623	0.245- 2.323
>= 3	Reference		

Table 2 revealed significant association between age and chlamydia trachomatis infection with p value of 0.008

TABLE 3: ASSOCIATION BETWEEN CHLAMYDIA DNA DETECTION AND INFERTILITY IN THE STUDY PARTICIPANTS.

Chlamydia DNA	Infertile n= 60(%)	Fertile n= 60(%)	Total n= 120(%)	P-value
Positive	6(10.0)	1(1.7)	7(5.8)	0.057
Negative	54(90.0)	59(98.3)	113(94.2)	
Total	60(50.0)	60(50.0)	120(100.0)	

Table 3 revealed no significant association between chlamydia DNA detection and infertility (p = 0.057)

TABLE 4: ASSOCIATION BETWEEN CHLAMYDIA DNA DETECTION AND TYPE OF INFERTILITY

Chlamydia DNA	Primary	Secondary	Total	P-value

	Infertility	Infertility		
Positive	1(4.8)	5(12.8)	6(10.0)	0.307
Negative	20(95.2)	34(87.2)	54(90.0)	
Total	21(35.0)	39(65.0)	60(100.0)	

Table 4 revealed there was no significant association between Chlamydia trachomatis infection and the type of infertility (P value =0.307).

TABLE 5: ASSOCIATION BETWEEN CHLAMYDIA DNA DETECTION AND TUBAL FACTOR INFERTILITY

Chlamydia DNA	Nonpatent tubes	Patent tubes	Total	P-value
Positive	3(7.7)	3(14.3)	6(10.0)	0.655
Negative	36(92.3)	18(85.7)	54(90.0)	
Total	39(65.0)	21(35.0)	60(100.0)	

Table 5 revealed no significant association between Chlamydia trachomatis infection and tubal factor infertility with p value of 0.655.

TABLE 6: DISTRIBUTION OF CHLAMYDIA DNA DETECTION BY PARTICIPANTS' CLINICAL DIAGNOSIS

	CHLAMYDIA DNA		P value
	Positive	Negative	
CLINICAL DIAGNOSIS			
Pelvic Endometriosis	0(0.0)	1(1.9)	0.776
Tubal factor	3(50.0)	17(31.5)	
Uterine factor	0(0.0)	3(5.6)	

Unknown	3(50.0)	33(61.1)
Total	6(10.0)	54(90.0)

Table 6 revealed there was no significant association between Chlamydia trachomatis infection and clinical diagnosis of infertility.