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Prevalence and Impact of *Chlamydia trachomatis* Infection in Primary and Secondary Infertile Women of Kurdish Ethnicity in Erbil Provence- Iraq

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Chlamydia trachomatis is the predominant bacterium transmitted through sexual activity, usually leading to symptomless infection and complications like infertility. Therefore, it is crucial to have an efficient diagnostic technique to minimize the long-term consequences of the disease. A case-control study in which 4 ml blood samples and endocervical swabs were collected from 134 infertile and 50 fertile Kurdish women. DNA was extracted from endocervical swabs using PCR to detect *C. trachomatis*. Also, the serological test was performed using the enzyme-linked immunosorbent assay (ELISA) test to estimate anti-chlamydial IgM from serum. Among the study participants, we found that from 134 infertile participants, Women with secondary infertility showed a higher prevalence, 86 (64.1%), than primary infertility, 48 (35.8%). The overall prevalence of genital *C. trachomatis*, determined through PCR testing, was found to be 62 cases (46.2%) among infertile women. Among these cases, 21 individuals (33.8%) were diagnosed with primary infertility, while 41 individuals (66.1%) had secondary infertility. In contrast, there were no positives among the fertile group. All fertile and infertile female groups were positive for anti-chlamydial IgM antibody by using ELISA. Still, the intensity of response differed between cases, with the highest rate observed in unexplained infertility (UI) (71.42%), followed by endometriosis (Endo) (66.66%), tubal factor infertility (TFI) (50%), and polycystic ovarian syndrome (PCOS) (37.5%). These results highlight the importance of considering *C. trachomatis* as a potential factor in infertility cases. The results concluded the highest frequency of chlamydial infection was among women with infertility than fertile women. PCR is the most reliable and exact diagnostic method for detecting it.

1.Introduction

Chlamydia trachomatis is a gram-negative obligate intracellular bacterium that belongs to the Chlamydiaceae family of Chlamydiae species and is responsible for one of the most widespread sexually transmitted infections (STIs) in the world and a significant contributor to infertility in women. It can evade the host's innate and adaptive immune systems by using autophagy (Rawre et al., 2017).

Chlamydial and Gonorrheal infection were the most frequent STIs that led to abnormal vaginal discharge in 2021 (Chayachinda et al., 2020). The World Health Organization (WHO) estimates that 129 million people worldwide have chlamydial infections in 2020 (Park et al., 2021).

Screening to identify chlamydial infections is necessary because most genital chlamydial infections are typically asymptomatic and can be transmitted to sexual partners (Rajabpour et al., 2020). If these infections are not treated, they can cause infertility and severe complications like pelvic inflammatory diseases (PID), TFI, early pregnancy termination, and increased susceptibility to ectopic pregnancy (Zhang et al., 2023).

Infertility is a significant global health issue, affecting millions of couples worldwide. It is defined as the inability to conceive after one year of unprotected sexual intercourse, and it can have profound emotional, social, and psychological consequences for individuals and couples (Mbah et al., 2022). Primary infertility was characterized as the incapacity to achieve pregnancy following consistent sexual intercourse without contraception for one year or more. On the other hand, secondary infertility was defined as the inability to conceive after engaging in regular sexual activity without contraception for six months or more despite having previously experienced a successful pregnancy (Abdu Alwadood, 2013). Infection with *C. trachomatis* is becoming more commonplace worldwide right now. Other than indirect approaches for detecting particular antibodies produced against *C. trachomatis*, diagnostic procedures for chlamydial infection detection differ depending on parameters, including culture, antigen testing, and molecular assays (Alkhuzai and Al-Shukr, 2022). The Diagnostic costs are much lower than treatment costs. As a result, early detection and screening may lower the incidence of gynecological infections and the cost of treating this condition (Azami et al., 2018). PCR is the most used test that responds positively after 5-7 days of *C. trachomatis* transmission (Sharief et al.,2021).

This study intends to close a knowledge gap about most *C. trachomatis* infections, particularly among primary and secondary infertile Kurdish women. We can better understand the burden of *C. trachomatis* and its possible influence on primary and secondary infertility rates by investigating the prevalence of *C. trachomatis* infection in this community.

2.Materials and Methods

Study Design

The study population consisted of 134 infertile women attending the Dr. Xawer infertility center at their reproductive age with primary and secondary infertility and 50 fertile women as control groups attending the family hospital station for vaccine plans for children who had no complaints of infertility. The inclusion criteria for the control group were the ability to become pregnant, a male fertility certificate, and 30 days without antibiotic use before the illnesses conditions (e.g., hypertension, thyroid dysfunction, diabetes mellitus, and cancer); (2) reproductive structure abnormality; (3) male factor infertility. Patients were categorized into four groups: TFI, PCOS, Endo, and UI (Wei et al., 2023).

Sample Collection

The medical team obtained endocervical swabs from infertile and control women and performed a regular gynecological per speculum examination to look for any symptoms of an infection; following cleaning, the bacterial transport swab (pink) was utilized. The swab was preserved in phosphate buffer saline and then centrifuged at 14000rpm for 20 min, and then the swab was removed after spinning. The sample was maintained in a freezer until DNA extraction and PCR were performed. From each group, 4 ml of venous blood was drawn using a disposable syringe, dispensed into a gel tube,

and allowed to solidify. Then, the serum was separated at room temperature using centrifugation. Serums were stored in a freezer to detect specific IgM by ELISA-circulating anti-chlamydial IgM antibodies in the serum of both the study and control groups.

DNA Isolation from Endocervical Swabs

Deoxyribonucleic acid was extracted from the 184 endocervical swabs using the BetaPrep genomic DNA extraction kit (Beta Bayern/German). The DNA extractions were performed as per the directions supplied by the manufacturer. The quantity and quality of DNA were determined using a NanoDrop® spectrophotometer. *C. trachomatis* was detected using the accurate Biosystems TM TaqMan® Assays.

PCR for Diagnosis of *C. trachomatis*

The polymerase chain reaction was performed on extracted DNA using the sequence of a primer supplied by Macrogen (Korea) and designed by (Mohammed and Al Fadhil, 2012) from the conserved region of the MOMP gene of *C. trachomatis*. Forward primer: 5'-CCTGTGGGGAAT GCTGCTGAA -3'and reverse primer: 5'-GTCGAAAACAAAGTCATCCAGTAGTA-3' to amplify a 144 bp DNA fragment. The reaction was performed in a final volume of 25 µl containing 0.5 µl of both primers, 12.5 µl of 2X Prime Taq Premix, and variable template DNA; then, the volume was adjusted with nuclease-free PCR water. In each experiment, a negative control was also used. The first denaturation was carried out for 5 min at 95 °C, followed by 35 cycles of 20 s each of denaturation at 94 °C, annealing at 57 °C for 45 s, and extension at 68 °C for 1 min. The last extension phase was performed for 10 minutes at 68 °C. The amplified products were run on 2% agarose gel, and the outcomes were recorded after being seen under a UV transilluminator. The positive control specimen had 144 bp repeat sequences, but the negative control sample did not, indicating that the reaction had been successfully performed.

Serology

A commercially available ELISA kit detected IgM antibodies against *C. trachomatis* in serum (Sunlong Biotech Co., China). The ELISA test gives a partially quantitative in vitro analysis to detect human antibodies of class IgM against *C. trachomatis*. Microtiter strips with eight break-off reagent wells coated with *C. trachomatis* antigens are included in the test kit. ELISA provides a reasonably quick and easy test for detecting Chlamydial infections in high-risk groups. ELISA kit analyses were performed according to established protocols from the manufacturer.

Statistical Data Analysis

The following equation was used: Cases/population size equals prevalence. P-values were considered significant at 0.05 and calculated using Graph-Pad Prism version 9.0 for all statistical analyses.

3.Results

Participant Characteristics

One hundred thirty-four infertile women and fifty control females were tested for current chlamydial infection by PCR, and ELISA collected serum samples to detect anti-chlamydial IgM. The age groups participating in the population study were between 20 and 45. The control group's primary and secondary infertility mean was 29.86 ±6.94, 32.63 ±7.42 and 31.77±6.44, respectively. Figure 1 showed no statistically significant variation among the groups, p = 0.11. (P>0.05).

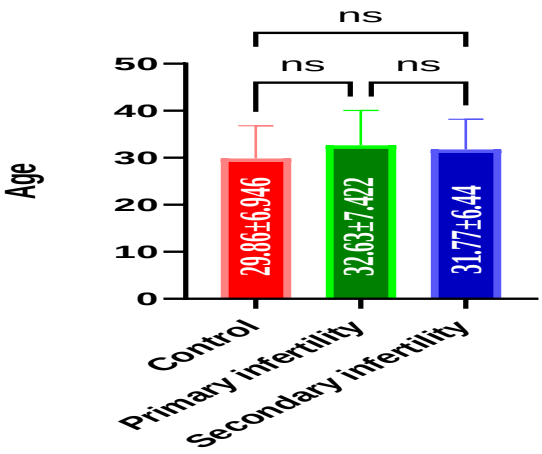

FIGURE 1: Age ranges represented in the population study

Our study found that from 134 infertile participants, 48 (35.8%) had primary infertility, and 86 (64.1%) revealed secondary infertility. As well as, within primary infertility, 8.3% of infertile women had Endo and TFI, 66.6% with PCOS,

and 16.6% appeared with unexplained infertility. Secondary infertile women with Endo, PCOS, TFI, and UI were 9.3%, 55.8%, 27.9%, and 6.9%), respectively, Table 1.

TABLE 1: frequencies of types of infertility according to etiological factors

Infertility types	Primary Infertility	Secondary Infertility	Total
Endo	4 (8.3%)	8 (9.3%)	12 (8.8%)
PCOS	32 (66.6%)	48 (55.8%)	80 (59.7%)
TFI	4 (8.3%)	24 (27.9%)	28 (20.8%)
UI	8 (16.6%)	6 (6.9%)	14 (10.4%)
Total	48 (35.8%)	86 (64.1%)	134

Detection of *C. trachomatis* by PCR

The overall prevalence of genital *C. trachomatis* by amplification of the MOMP gene was (62/134) 46.26 % in infertile women, 21 (33.87%) of them with primary infertility and 41 (66.12%) with secondary infertility. In contrast, there were no positives among the fertile group. The expected fragment size of 144 bp was revealed by agarose gel electrophoresis, as shown in Table 2 and Figure 2.

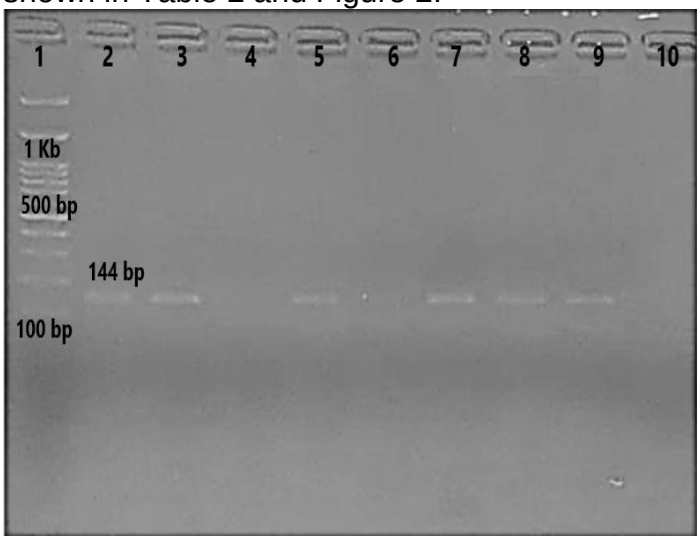

FIGURE 2: PCR amplification of MOMP gene of *C. trachomatis*.The first lane indicates a 100 bp DNA ladder; lanes 2, 3, 5, 7, 8, and 9 indicate positive *C. trachomatis* samples; lanes 3 and 5 indicate negative *C. trachomatis* samples; lane 10 indicates negative control.

The correlation between each specific cause of infertility and positive *C. trachomatis* PCR results was indicated in Table 3 and Figure 3. The PCOS as a cause of infertility in the studied population was the highest 80/134 (59.7%); it suggests that among the individuals in the case group, the positive result was highest for UI (71.42%), followed by Endo (66.66%), TFI (50%), and PCOS (37.5%).

TABLE 2: Detection of *C. trachomatis* by PCR assay

Infertility Type	Positive *C. trachomatis*	Negative *C. trachomatis*	Total
Primary Infertility	21 (33.87%)	27 (37.5%)	48 (35.82%)
Secondary Infertility	41 (66.12%)	45 (62.5%)	86 (64.17)
Total	62 (46.26%)	72	134

TABLE 3: Cause of infertility in the case group, with correlation to *C. trachomatis* PCR results

Infertile	No. of	Positive PCR
Endo	12 (8.95%)	8 (66.66%)
PCOS	80 (59.7%)	30 (37.5%)
TFI	28 (20.89%)	14 (50%)
UI	14(10.44%)	10 (71.42%)
Total	134	62

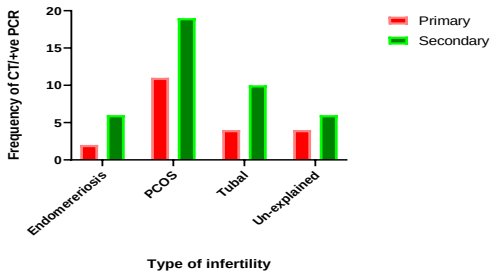

FIGURE 3: Cause of infertility in the case group, with correlation to positive *C. trachomatis* PCR results

Evaluation of Serum Anti-chlamydial IgM Antibody between Groups

The ELISA method was used to analyze the prevalence of serum IgM antibodies specific to *C. trachomatis* in infertile and fertile women. All groups of infertile females tested positive for anti-chlamydial IgM antibody through ELISA, although the strength of the response varied among cases. The mean

values of anti-chlamydial IgM were as follows: (2.38 ± 1.89, 0.63 ± 0.25, 0.85 ± 0.25, and 0.61 ± 0.22) pg/ml in the Endo, PCOS, TFI, and UI groups, respectively. The mean value of anti-chlamydial IgM in the control group was 0.32 ± 0.17. Statistical analysis revealed significant differences in this factor between the endometriosis group and the PCOS, TFI, and UI groups. However, no significant differences were observed within the other groups, as depicted in Figure 4.

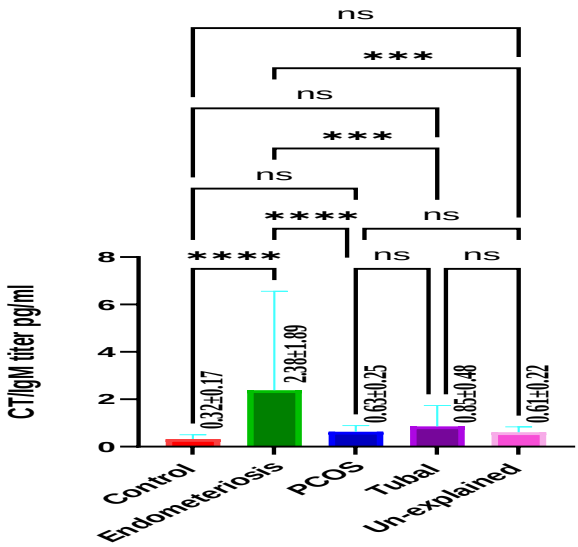

FIGURE 4: Evaluation of serum anti-chlamydial IgM and their correlation with women infertile groups

Assessing the Serum Anti-Chlamydial IgM and C. trachomatis PCR Results in Infertile Women

The average *C. trachomatis* IgM level was consistently higher in the positive *C. trachomatis* group compared to the negative group across all conditions studied. This finding strongly supports the use of swab-based PCR as a diagnostic tool for *C. trachomatis* infection, as depicted in Figure 5. In the Endo group, the mean *C. trachomatis* IgM level was 0.39±0.08 pg/ml in the negative group and 3.38±2.98 pg/ml in the positive group. Similarly, in the PCOS group, the mean C. trachomatis IgM level was 0.54±0.16 pg/ml in the negative group and 0.77±0.31 pg/ml in the positive group. Additionally, in the TFI group, the mean *C. trachomatis* IgM level was 0.46±0.06 pg/ml in the negative group and 1.25±1.03 pg/ml in the positive group. Lastly, in the UI group, the mean *C. trachomatis* IgM level was 0.46±0.07 pg/ml in the negative group and 0.67±0.23 pg/ml in the positive group.

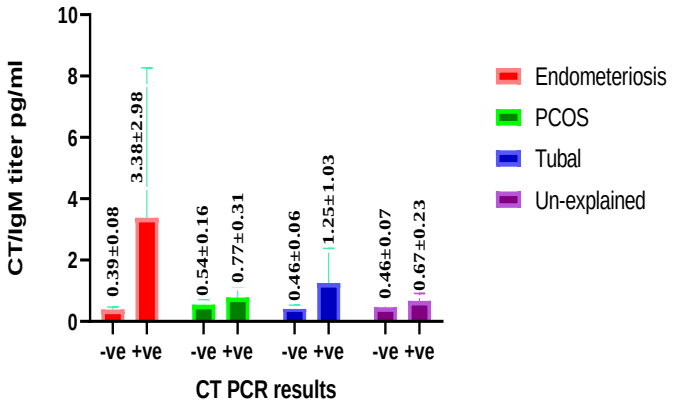

FIGURE 5: Correlation between serum anti-chlamydial IgM and *C. trachomatis* PCR results in infertile women

3.Discussion

There is limited data on the incidence of chlamydial infection, particularly in underdeveloped nations, including Iraq, where standard laboratory testing for this disease is inaccessible (Gahanbarzade et al., 2021).

The age groups participating in the population study were between 20 and 45. The control group's primary and secondary infertility mean was 29.86 ±6.94, 32.63 ±7.42 and 31.77±6.44, respectively. Our result aligns with Ajani et al. (2017); they demonstrate that the women's mean age was 33.98 ± 3.85, with a range of 24 to 40 years, which falls beyond the range of reproductive age. The late initiation of sexual activity in our group may be one reason. The age at which sexual activity begins and *C. trachomatis* infection is strongly correlated (Mawak et al., 2011). In our study, the prevalence of primary infertility was 35.8%. In comparison, secondary infertility accounted for 64.1% of cases, and *C. trachomatis* was detected by PCR in 62 out of 134 participants, accounting for 46.26% of the cases. Furthermore, 21 (33.87%) had primary infertility among those who tested positive for Chlamydial infection, while 41 (66.12%) had secondary infertility. However, the type of infertility was not found to be statistically significant for *C. trachomatis* infection.

Our results agree with studies conducted in Iraq; Sahi and Mohammed (2020) in Basra revealed

that the prevalence of *C. trachomatis* infection in women with infertility was 43.2%, and 37.2% of them with primary infertility and 62.8% with secondary infertility; Salmanet al., (2018) in Kirkuk demonstrated that the prevalence of *C. trachomatis* infection in women with PID was 41.96%, and Tahir, (2016) and (Shariefet al., 2021) in Basra they reported a slightly higher prevalence 48% for *C. trachomatis*.

The lower prevalence rate of *C. trachomatis* reported by (Abdu Alwadood, 2013) and (Agha et al., 2011) through using the PCR technique in patients with infertility was 29.3% and 25.7% in Egypt, 32% in Iran (Marashi et al., 2014); In Iraq 22% and 17.07% by (Ali and Shia, 2018), (Shamkhi et al., 2022)respectively in Baghdad and 11.33% by Haider, (2022) in Zakho. In the United Arab Emirates, 10.4% (Mehrabani et al., 2014) and India,13.5% (Dhawan et al., 2014).

There is a significant difference in infection rates between the two groups. Our study's infection rates varied from previous research, which may be attributed to certain limitations, including the sample size, economic and environmental variables, specimen-collecting methods, diagnostic-based methods, and targeted genes.

The results of the present study indicate that PCOS was identified as the leading cause of infertility. Additionally, the percentage of positive *C. trachomatis* PCR results varied among different causes of infertility, with the highest rate observed in UI (71.42%), followed by Endo (66.66%), TFI (50%), and PCOS (37.5%). These results agree with (Deshpande and Gupta, 2019) and (Mousa et al., 2019), who found that the leading cause of infertility was the ovarian cause of PCOS and TFI while disagreeing with Abdu Alwadoodet al. (2013), revealed TFI as a cause of infertility in the studied case group was the highest cause of infertility (38.6%). (Siemer et al., 2008) reported a prevalence of 39.3% in Ghana, while 36% was reported in Egypt among patients with UI.

It has been proposed that chlamydial infection may reduce fertility by causing an inflammatory reaction to produce anti-sperm antibodies (ASA) that might impede sperm movement and prevent sperm-ovum contact. Additionally, it has been suggested that the antigens of *C. trachomatis* and spermatozoa may already be reactive (Abdella et al., 2015). The variation in C. trachomatis PCR results among different causes of infertility could be attributed to several factors: underlying Pathophysiology: each cause of infertility, such as PCOS, Endo, TFI, or UI, has distinct underlying pathophysiological mechanisms. For instance, PCOS is characterized by hormonal imbalances and metabolic abnormalities (Deshpande and Gupta, 2019). Another explanation for this is that chlamydial infections are asymptomatic; these undetected or subclinical infections may persist for an extended period and can lead to complications, including infertility (Siam and Hefzy, 2012).

In this study, all fertile and infertile groups tested positive for anti-chlamydial IgM using an ELISA test, but the intensity of response significantly differed between cases. The elevated prevalence of false positive reactions in the ELISA test may be related to cross-reactivities reported among *C. trachomatis* and other *Chlamydia* species, such as *C. pneumoniae* and *C. psittaci* (Baud et al., 2010).

Since swab-based PCR is the most sensitive and specific non-culture diagnostics, we employed it to identify chlamydial infections in infertile women (Sahi and Mohammed, 2020); the PCR technique is widely recognized as the most reliable method for STI diagnosis (Kebbi-Beghdadi et al., 2022).

Additionally, social and cultural factors that hinder women from reporting sexual symptoms, limited access to testing facilities in numerous healthcare centers, and the predominantly symptomless nature of the infection can contribute to elevated levels of undiagnosed chlamydial infections and antibodies. Compared to PCR, ELISA and antigen detection tests exhibit lower sensitivity and specificity in detecting *C. trachomatis*. Consequently, PCR has been established as the preferred method for identifying asymptomatic infections, which is why it was selected as the gold standard for this study (Ajani et al., 2017).

The mean *C. trachomatis* IgM level in the positive *C. trachomatis* group is higher than that in the negative *C. trachomatis* group, which

suggests using *C. trachomatis* PCR as a diagnostic tool for *C. trachomatis* infection. Swab-based PCR is a molecular test that detects the presence of specific DNA sequences and is considered highly sensitive and specific for detecting *C. trachomatis* (Bébéar and De Barbeyrac, 2009).

Sahi and Mohammed (2020) observed that serologic evaluations tend to be ineffective in determining the presence of genital tract infection caused by *C. trachomatis*; due to the strong background of anti-chlamydial antibodies or seronegativity, the serological test is generally unreliable in diagnosing chlamydial infection. Our findings were consistent with active disease.

4.Conclusion

Studies about the prevalence of *C. trachomatis* infection in the Kurdish population are limited. These findings suggest that *C. trachomatis* infection may be associated with primary and secondary infertility. Still, the type of infertility does not significantly affect the likelihood of *C. trachomatis* infection. These results highlight the importance of considering *C. trachomatis* as a potential factor in cases of infertility, regardless of the type. The results concluded that infertile women had a higher prevalence of chlamydial infection than fertile women. Secondary infertility among women was more common compared to primary infertility. Based on our research, it is strongly advised to test infertile women for *C. trachomatis*. Swab-based PCR is the technique that most often detects *C. trachomatis* transmission.

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Conflict of interest

The authors declared no conflict of interest, it's part of the PhD thesis.

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