Reports of Biochemistry & Molecular Biology
Vol.12, No.4, Jan 2024

Original article

www.RBMB.net

Interleukin-10 Gene Polymorphisms Modulate the Risk of Infertility in *Chlamydia trachomatis* Positive Kurdish Women in Erbil Province

Lawin Ahmed Omar*[1], Suhaila Nafee Darogha[1]

Abstract

Background: There is evident inter-individual variability in women's responses to Chlamydial infections and reproductive tract problems. Women's genetic variations within the Interleukin-10 (IL-10) gene have been linked to variances in response to *Chlamydia trachomatis* infection. This study was aimed to demonstrate the profound association of IL-10 with infertility and demonstrate the role of IL-10 (-592 C/A rs1800872) and (-1082 A>G rs1800896) single nucleotide polymorphism (SNPs) gene in the susceptibility and severity of a *C. trachomatis* infection.

***Method*:** In this evaluation study, serum IL-10 concentration was measured in 134 women diagnosed with infertility and 50 healthy volunteers by enzyme-linked immunosorbent assay (ELISA). The tetra-amplification refractory mutation system-PCR (T-ARMS-PCR) analysis was performed to detect the genotyping of the rs1800872 and rs1800896 SNPs genes.

***Result*:** Both female groups were positive for anti-chlamydial IgM antibody, but the intensity of response differed between cases. At the same time, the incidence of genital *C. trachomatis* by PCR was 46.2% in infertile women. The serum concentration of IL10 was lower in infertile women than healthy participants and higher in infertile *C. trachomatis*-positive women compared to infertile *C. trachomatis*-negative in all groups except endometriosis (Endo) infertility. In rs1800872, the CA genotype and C allele are associated with an increased risk for infertility, except in polycystic ovarian syndrome (PCOS), which is an A allele. In the case of rs1800896, the AG genotype and G allele show a greater risk for infertility.

***Conclusions*:** Our results confirmed that rs1800872 and rs1800896 gene polymorphisms were associated with an increased risk of *C. trachomatis* infection.

Keywords: *Chlamydia trachomatis*, Female infertility, IL-10, SNP.

Introduction

Chlamydia trachomatis is an obligate intracellular bacterium that attacks the epithelial tissues of the genital tract. Numerous serovars, including A-C, D -K, and L1 -L3 serovars, are related to trachoma, urogenital disease, and invasive lymphoma granuloma venereum (LGV), respectively (1). Chlamydial infection is among the most common bacterial transmissible through sexual contact worldwide (2). Repeated infections can cause irreversible tissue damage, including cervicitis, salpingitis, pelvic inflammatory disease, and infertility. The infections are often asymptotic, and reinfections are frequent (3). Cell-mediated immune responses are crucial for infection management due to Chlamydia's intracellular lifestyle (4).

Host genetics can account for many variations in how individuals respond to a *C. trachomatis* infection. According to related research, the host's genetic makeup may account for about 40% of the immune response to *C. trachomatis* infection

1: Department of Biology, College of Education, Salahaddin University-Erbil, Erbil City, Kurdistan Region, Iraq.
***Corresponding author: Lawin Ahmed Omar; Tel: +96 47505830181; E-mail: Lawin.omar@su.edu.krd.**
Received: 18 Aug, 2023; Accepted: 28 Jan, 2024

[DOI: 10.61186/rbmb.12.4.550]

variation. Various SNPs have been associated with variations in *C. trachomatis* sensitivity and severity (5).

The significant SNPs for *C. trachomatis* are intra- and extra-cellular pathogen recognition receptors (PRRs), cytokines, and chemokines associated with and influencing the immune system's response after *C. trachomatis* infection. Some SNPs increase the risk of *C. trachomatis* infection or complications after infection (6).

Interleukin-10 is a multifunctional cytokine (7); the primary activities of IL-10 include limiting and terminating inflammatory responses, inhibiting the release of pro-inflammatory cytokine, and modulating immune cell differentiation and proliferation (8). IL-10 is considered a key cytokine mediating *C. trachomatis*-specific immune responses. Immunosuppressive IL-10 may reduce pathology in the upper reproductive tract. Still, when produced in excess or at the wrong time, it can also impede the removal of pathogens and cause chronic infection. Variation between individuals in *C. trachomatis*-specific immune responses depends on the IL-10 -1082 SNP (4).

The interleukin-10 gene is located on chromosome 1q31-32, and gene polymorphisms have been extensively studied concerning various infectious and inflammatory diseases. The three most commonly studied IL-10 gene polymorphisms are -592 C/A, -819 C/T, and -1082 G/A, which are located in the promoter region of the IL-10 gene and can affect the production and activity of IL-10 (9). IL-10 gene polymorphisms, specifically the -1082 A/G (rs1800896) polymorphism, have been shown to modulate the outcomes of a *C. trachomatis* infection (10). Individual differences in immunological responses to *C. trachomatis* depend on the IL-10 -1082 SNP and contribute to differences in tubal damage susceptibility and severity (11).

Our study aims to determine the prevalence of *C. trachomatis* and investigate the association between IL-10 gene polymorphisms and the risk of infertility in *C. trachomatis*-positive Kurdish women in Erbil province.

Materials and Methods

Study participants

A cross-sectional, case-control study was conducted on volunteers who attended Dr. Xawer Center for Infertility and Ashti Hospital in Erbil City from September 2021 to September 2022. The study comprised two groups:

The **control group** included 50 (27.17%) fertile women; the exclusion criteria lacked a history of fertility problems and current antibiotic therapy, and their ages ranged between 21-44 years.

Infertile group: 134 (72.82%) patients. The criteria for inclusion in the exhausted group were the lack of ability to become pregnant despite attempting for at least a year, confirmation of fertility from men, and the absence of antibiotic treatment within 30 days before this evaluation, and their ages ranged between 20-45 years. The causes of female infertility were the involvement of PCOS, tubal factor infertility (TFI), Endo, and unexplained infertility (UI).

Ethical concerns were addressed by fully informing volunteers about study objectives and prioritizing ethics. Participants voluntarily signed informed consent. Patients were divided into four categories based on infertility cause, with medical history or standard fertility work-up determining the cause (12).

Blood and Swab collection

7 ml of venous blood was collected and divided into two aliquots. The first aliquot was centrifuged to measure IL-10 and IgM antibodies, while the second aliquot was frozen for genomic research. Using the PCR method, a gynecologist obtained an endocervical swab from the endocervical canal to identify the DNA of *C. trachomatis*.

Detection of Serum IL-10 and Recent anti-Chlamydial Antibodies

After thawing the frozen serum at room

[DOI: 10.61186/rbmb.12.4.550]

temperature, the commercial ELISA kit performed serum IL-10 and anti-chlamydial IgM concentrations (Sunlong Biotech Co., China, Lot No:20220215, REF: SL1734Hu). Both ELISA kit analyses were performed according to established protocols from the manufacturer.

DNA Extraction and Molecular Analysis of C. trachomatis

The nucleic acid of *C. trachomatis* was extracted from the endocervical swabs using a BetaPrep genomic DNA extraction kit (Beta Bayern/German, Cat. No: MDE 101)) according to the manufacturer's directive. DNA was quantified using nano-drop (Biotek) and run in 1.5% gel agarose electrophoresis. A thermocycler was used for a conventional PCR reaction and amplification of the MOMP gene sequence (Macrogen, South Korean); the forward and reverse primers for detecting *C. trachomatis,* as shown in Table 1, were designed by (13). The reaction was carried out with a total volume of 25 µl, which included 0.5 µl of each primer and 12.5 µl of 2X Prime Taq Premix (Genet Bio, Korea, product code: 35001), variable template DNA then, the solution's volume was adjusted with nuclease-free PCR water. In each experiment, a negative control was run. The PCR reaction protocols were as follows: a 30-second initial denaturation at 94 ℃ followed by 35 cycles of 20-second denaturation at 94 °C, 45-second annealing at 57 °C, and 1-minute elongation at 68 °C. The last elongation phase was performed for 10 minutes at 68 °C. The amplicon was run on 2% agarose gel and analyzed under a UV transilluminator.

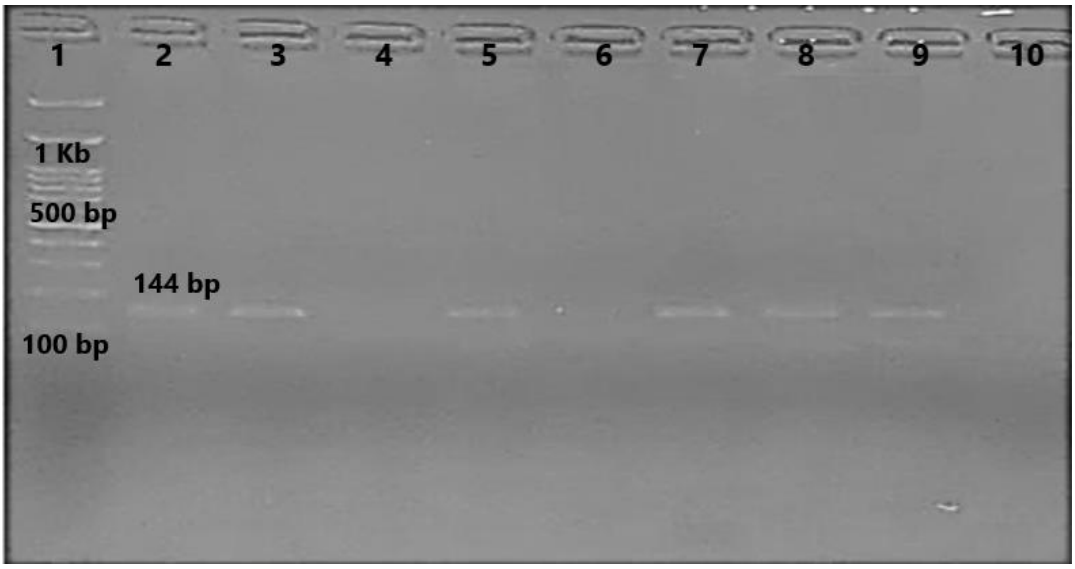

Fig. 1. PCR amplification of MOMP gene of *C. trachomatis*. The first lane indicates a 100 bp DNA ladder; lanes 2, 3, 5, 7, 8, and 9 indicate positive *C. trachomatis* samples; lanes 3 and 5 indicate negative *C. trachomatis* samples; lane 10 indicates negative control.

IL -10 Gene Polymorphisms

The IL10 -592 A>C (rs1800872) and -1082 A>G (rs1800896) genotypes were determined by tetra ARMS-PCR. Genomic DNA was isolated from blood using the Genomic DNA Extraction kit (Beta Bayern, Germany), quantified using nano-drop (Biotek), and run in 1% gel agarose electrophoresis. Specific primers are shown in Table 1 (Macrogen, South Korean). The PCR reaction was conducted using 2X Prime Taq Premix (Genet Bio, Korea), with a 25 µl reaction mixture containing 1 µl of genomic DNA, 1 µl of each primer, 12.5 µl of 2X Taq master Mix with standard buffer, and 7.5 µl of Nuclease-free water. A negative control, including water rather than DNA, was introduced to each pair of assays to check for contamination. The PCR profile consisted of an initial melting step of 5 minutes at 95 °C, followed by 35

[DOI: 10.61186/rbmb.12.4.550]

cycles of 30 s of denaturation at 95 °C, annealing at 58 °C for -592 A>C and 65 °C for -1082A>G for 30 s, elongation at 72 °C for 1 min, and a final elongation at 72 °C for 10 min. The amplicon length determined each SNP's genotype after gel electrophoresis on 2% agarose gel stained with 0.01 µg/ µl safe DNA gel stain (Figs. 2 & 3).

Table 1. The primer sequence was obtained and produced by Macrogen (Korea).

MOMP Gene				
Forward	CCTGTGGGGAATGCTGCTGAA			
Reverse	GTCGAAAACAAAGTCATCCAGTAGTA			
IL-10 Gene (Gene ID: 3586)				
	(-1082 A /G) (rs1800896)	**Size (bp)**	**(-592 C /A) (rs1800872)**	**Size (bp)**
Forward outer	CTCCCAGTTACAGTCTAAACTGGAATG	433	TAATGAAATCGGGGTAAAGGAGCCTAGC	425
Reverse outer	GGATTAAATTGGCCTTAGAGTTTCTTTT	433	GTACAGGCGGGGTCACAGGATGTATT	425
Forward inner	**G Allele** AAGACAACACTACTAAGGCTTCTTTGGTAG	199	**C Allele** CTCAGTTGGCACTGGTGTACCCTTGTAC	211
Reverse inner	TTTCCTCTTACCTATCCCTACTTCCACT	292	**A Allele** TGGGATGAATACCCAAGACTTCTCCTTG	268

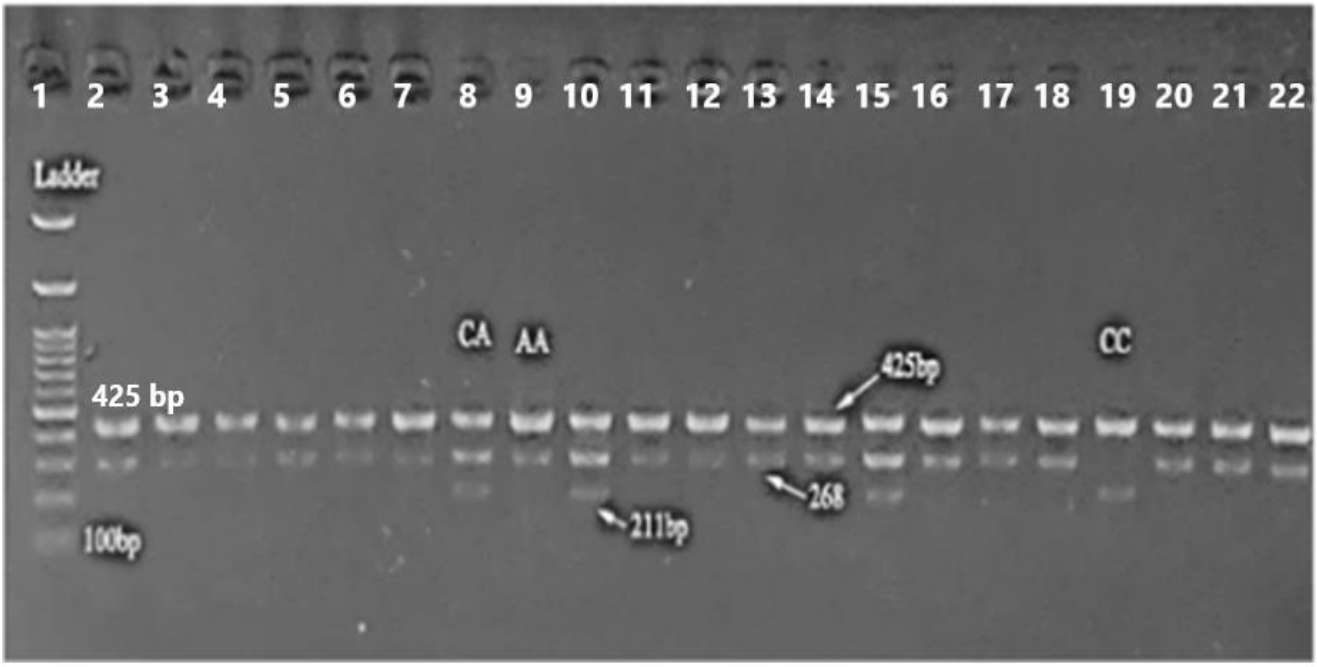

Fig. 2. Amplification bands of the different genotypes of SNP rs1800872: first lane 100 bp DNA ladder; lanes 2-7, 9, 11-14, 16-18, 20-22 indicate AA genotype; lanes 8, 10, 15 indicate CA genotype; lane 19 indicate CC genotype. Genotyping interpretation (CC: N: 211, 425 bp; CA: HE: 211, 268,425 bp; AA: MH: 268, 425 bp). NH: Normal homozygote; HE: Heterozygote; MH: Mutant homozygote.

[DOI: 10.61186/rbmb.12.4.550]

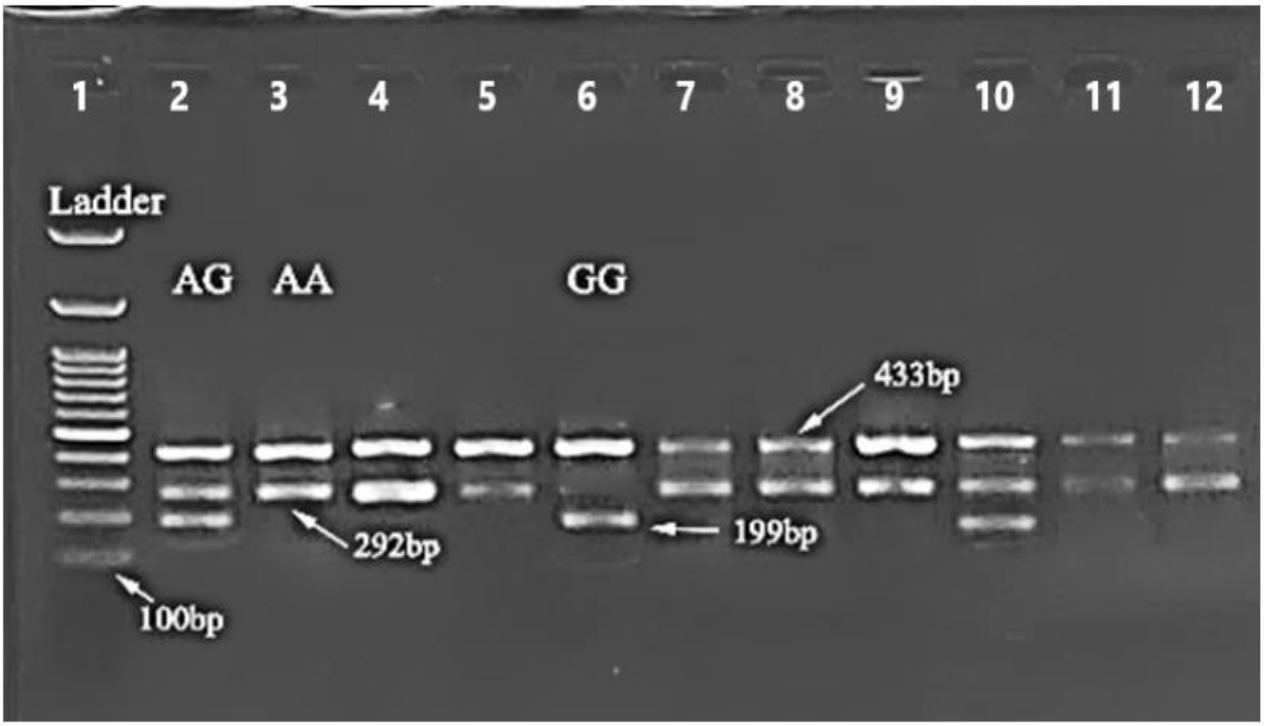

Fig. 3. Amplification bands of the different genotypes of SNP rs1800896: first lane 100 bp DNA ladder; lanes 2, 10 indicate AG genotype; lanes 3-5, 7-9, 11, 12 indicate AA genotype; lane 6 indicate GG genotype. Genotyping interpretation (AA: NH: 292, 433 bp; AG: HE: 199, 292, 433 bp; GG: MH: 199, 433 bp). NH: Normal homozygote; HE: Heterozygote; MH: Mutant homozygote.

Statistical analysis

Graph-Pad Prism version 9.0 was used to conduct all statistical analyses. The odds ratio (OR), relative risk, and 95% confidence intervals (CI) were estimated to determine the relationship between genotypes and infertility. Statistics were judged to be significant for P-values under 0.05.

Results

One hundred eighty-four volunteers enrolled in the current study. The mean age for control was 29.86±29.86, while for infertile patients as Endo, PCOS, TFI, and UI, were 33.16±6.93, 30.21±6.46, 34.71±5.5, and 36.50±7.32, respectively. Significant differences were found in the mean ages of the control and patient groups P = 0.000.

Evaluation of serum anti-chlamydial IgM antibody between groups

The prevalence of serum IgM antibodies specific to *C. trachomatis* in infertile women was analyzed using ELISA. All infertile female groups were positive for anti-chlamydial IgM antibody, but the intensity of response differed between cases. The mean values of anti-chlamydial IgM were: (2.38 ±4.17, 0.85 ±0.88, 0.63 ±0.25, and 0.61 ±0.22) pg/ml in (Endo, TFI, PCOS, and UI), respectively. The statistical analysis shows significant differences between the control and infertile groups, while there are non-significant differences within groups (Fig. 4.)

[DOI: 10.61186/rbmb.12.4.550]

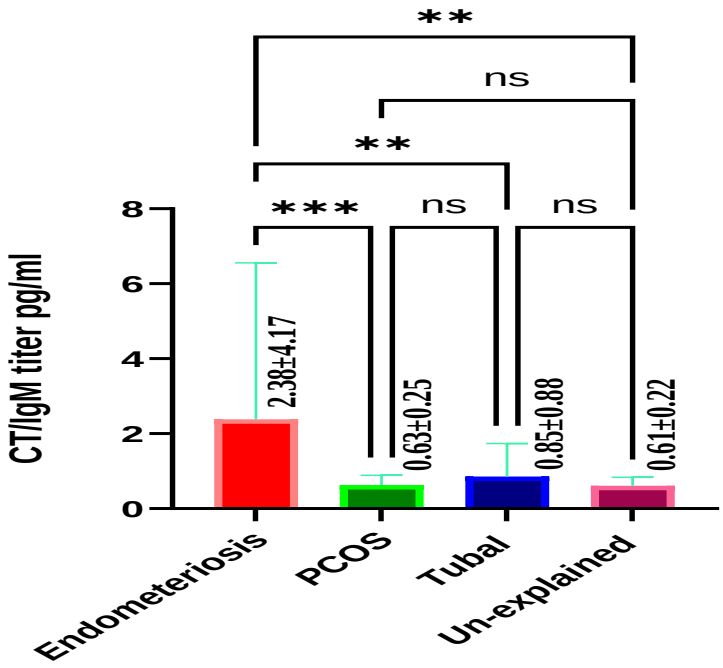

Fig. 4. Mean distribution of serum anti-chlamydial IgM with standard deviation and their correlation with Endo, PCOS, TFI, and UI women infertile groups. *P < 0.05 was considered statistically significant; ns indicate non-significant.

Detection of C. trachomatis by PCR

The overall prevalence of genital *C. trachomatis* by amplifying the MOMP gene was (62/134) 46.26 % in infertile women. In contrast, there were no positives among the fertile group. The expected fragment size of 144 bp was observed by agarose gel electrophoresis (Fig. 1)

Table 2 indicates the correlation between each specific cause of infertility and positive *C. trachomatis* PCR results. The PCOS as a cause of infertility in the studied population was the highest 80/134 (59.7%), while the positive PCR result was highest for UI (71.42%), followed by Endo (66.66%), TFI (50%), and PCOS (37.5%).

Table 2. Correlation between the cause of infertility in the case-cohort and the *C. trachomatis* PCR results

Infertile Groups	No. of Cases	Positive PCR Results
Endometriosis	12 (8.95%)	8 (66.66%)
PCOS	80 (59.7%)	30 (37.5%)
TFI	28 (20.89%)	14 (50%)
Unexplained infertility	14(10.44%)	10 (71.42%)
Total	134	62 (46.26%)

Assessing the serum anti-chlamydial IgM and C. trachomatis PCR results in infertile women

The mean *C. trachomatis* IgM level in the positive *C. trachomatis* group is higher than in the negative *C. trachomatis* group for all infertile groups: (3.38±4.88 pg/ml, 0.77±0.31 pg/ml, 1.25±1.13 pg/ml, and 0.67±0.23 pg/ml in Endo, PCOS, TFI, and UI respectively). Moreover, the mean levels of anti-chlamydial IgM for negative *C. trachomatis* were: 0.39± 0.08 pg/ml; 0.54± 0.16 pg/ml; 0.46± 0.06 pg/ml and 0.46± 0.07 pg/ml in Endo, PCOS, TFI, and UI respectively (Fig. 5).

[DOI: 10.61186/rbmb.12.4.550]

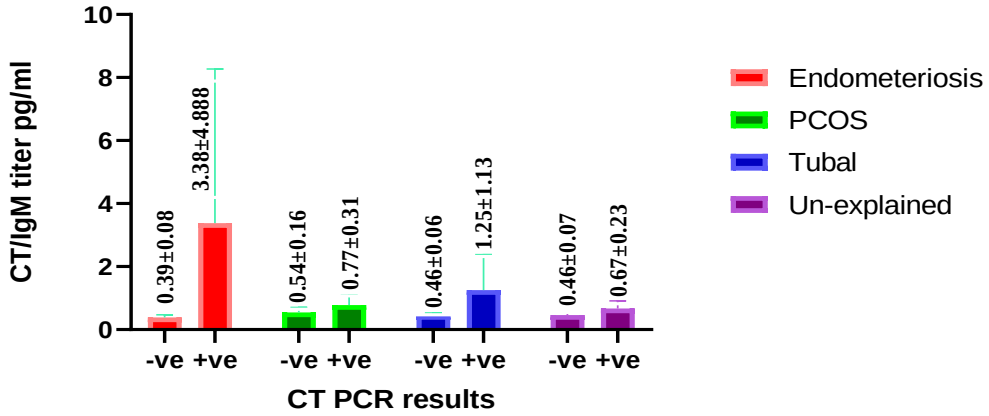

Fig. 5. Mean distribution of serum anti-chlamydial IgM with standard deviation and *C. trachomatis* PCR results in Endo, PCOS, TFI, and UI women infertile groups; –ve indicate negative *C. trachomatis* group, +ve show negative *C. trachomatis* group.

Evaluation of serum IL-10 and their correlation within groups

The IL-10 was estimated in the serum of all participants, and the mean values were as healthy: IL-10 (– 1082) =308.7 pg/ml; Endo: IL-10 =73.54 pg/ml: PCOS: IL-10= 184.6 pg/ml; TFI: IL-10 = 222.0 pg/ml and UI: IL-10= 291.9 pg/ml. The statistical analysis shows a declining level of serum IL-10 in infertile participants. Significant differences were observed in this factor between healthy and Endo and PCOS participants but not with TFI and UI participants (Fig. 6).

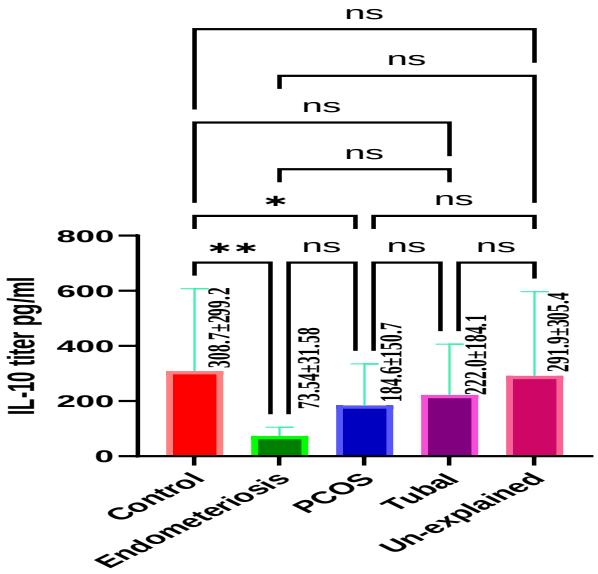

Fig. 6. Mean distribution of serum IL-10 with standard deviation among fertile and infertile participants; *P < 0.05 was considered statistically significant; ns indicate non-significant.

Associations of IL-10 (rs1800872 and rs1800896) genotypes and allele frequency with women's infertility

The distribution of genotypes and alleles frequency of both SNPs among the patients and the controls showed substantial differences (p < 0.001). The rs1800872 SNP was found to be associated with a higher risk of infertility in women with the CA genotype, were: (50%, 37.5%, 39.28%, and 42.85%) and 14% for infertile Endo, PCOS, TFI, UI, and controls, respectively. The rs1800872 was also associated with an increased risk for infertility in the recessive (CC+CA) vs. AA model for

[DOI: 10.61186/rbmb.12.4.550]

Endo, PCOS, TFI, and UI, with significant differences in Endo and PCOS. The frequencies of major allele A compared with the minor allele C for cases were (66.6%, 73.7%, 73.2%, 71.4%), (33.3%, 26.2%, 26.7%, 28.5%) in (Endo, PCOS, TFI, and UI) infertile groups respectively, and 89 % and 11% in controls respectively. The rs1800872 was also associated with an increased risk for infertility in the recessive model (CC+CA) vs. the AA model for Endo, PCOS, TFI, and UI, with significant differences in Endo and PCOS, as shown in Table 3.

On the other hand, the distribution of IL-10 rs1800896 SNP genotypes between cases and healthy individuals was significantly different: 58.33%, 65%, 64.28%, and 71.41% of Endo, PCOS, TFI, and UI case groups, respectively and 92% of healthy controls carried the AA genotype, while AG and GG genotypes were: 33.3%, 30%, 28.57%,21.41% and 8.3%, 5%, 7.14%, 7.14 for Endo, PCOS, TFI and UI infertile groups respectively and 6% and 2% for controls. Major allele A was associated with an increased risk of women's infertility. The dominant model (GG + AG) vs. AA is associated with an increased risk for women infertility in Endo infertility PCOS, TFI, and UI groups. The results were statistically significant except in UI groups. Also, the C allele is associated with an increased risk for infertility in Endo, as shown in Table 4.

Table 3. The genotypes, genetic models, and allele frequency of IL-10 (-592C/A) in infertile women.

Infertility categories	IL-10 (-592C/A) (rs rs1800872)	Infertile female frequency	Control female frequency	Relative Risk	Etiology or Preventive Fraction	Exact Fishers Probability	95% Confidence Intervals
			Genotype				
Endo	CC	1 (8.33%)	2 (4%)	2.18	0.04	0.482	0.20-24.26
	CA	6 (50%)	7 (14%)	6.14	0.41	0.013*	1.61-23.49
	AA	5 (41.66%)	41 (82%)	0.16	0.69	0.008**	0.04-0.58
	(CC+CA) vs. AA	7 (58.33%)	9 (18%)	3.39	0.27	0.05*	1.06-10.83
	(AA+CA) vs. CC	11 (91.66%)	48 (96%)	0.29	0.59	0.05*	0.09-0.94
	C allele	8 (33.33%)	11 (11%)	4.05	0.25	0.011*	1.43-11.43
	A allele	16 (66.66%)	89 (89%)	0.25	0.67	0.011*	0.09-0.70
PCOS	CC	6 (7.5%)	2 (4%)	1.95	0.03	0.710	0.38-9.89
	CA	30 (37.5%)	7 (14%)	3.69	0.27	0.005**	1.48-9.15
	AA	44 (55%)	41 (82%)	0.27	0.60	0.002**	0.12-0.62
	(CC+CA) vs. AA	36 (45%)	9 (18%)	2.59	0.20	0.027*	1.15-5.83
	(AA+CA) vs. CC	74 (93.75%)	48 (96%)	0.39	0.51	0.027*	0.17-0.87
	C allele	42 (26.25%)	11 (11%)	2.88	0.17	0.003**	1.41-5.89
	A allele	118 (73.75%)	89 (89%)	22.73	0.70	0.000***	11.12-46.48
TFI	CC	2 (7.14%)	2 (4%)	1.85	0.03	0.615	0.25-13.49
	CA	11 (39.28%)	7 (14%)	3.97	0.29	0.026*	1.34-11.78
	AA	15 (53.57%)	41 (82%)	0.25	0.61	0.017*	0.09-0.70
	(CC+CA) vs. AA	13 46.42%)	9 (18%)	2.67	0.20	0.080	1.02-6.99
	(AA+CA) vs. CC	26 (92.85%)	48 (96%)	0.38	0.52	0.052	0.14-0.98
	C allele	15 (26.78%)	11 (11%)	2.96	0.17	0.014*	1.26-6.96
	A allele	41 (73.21%)	89 (89%)	0.34	0.58	0.014*	0.14-0.79
UI	CC	1 (7.14%)	2 (4%)	1.85	0.03	0.530	0.17-20.53
	CA	6 (42.85%)	7 (14%)	4.61	0.33	0.028*	1.27-16.75
	AA	7 (50%)	41 (82%)	0.22	0.64	0.031*	0.06-0.076
	(CC+CA) vs. AA	7 (50%)	9 (18%)	2.87	0.22	0.107	0.92-8.98
	(AA+CA) vs. CC	13 (92.85%)	48 (96%)	0.35	0.54	0.107	0.11-1.09
	C allele	8 (28.57%)	11 (11%)	3.24	0.19	0.033*	1.17-8.96
	A allele	20 (71.42%)	89 (89%)	0.31	0.61	0.033*	0.11-0.89

[DOI: 10.61186/rbmb.12.4.550]

Table 4. The genotypes, genetic models, and allele frequencies of IL-10 (− 1082 A /G) in the infertile women.

Infertility categories	IL-10 (-1082A/G) (rs rs1800896)	Infertile female frequency	Control female frequency	Relative Risk	Etiology or Preventive Fraction	Exact Fishers Probability	95% Confidence Intervals
			Genotype				
Endo	AA	7 (58.33%)	46 (92%)	0.12	0.8	0.010*	0.03-0.54
	AG	4 (33.3%)	3 (6%)	7.83	0.29	0.022*	1.54-39.96
	GG	1 (8.3%)	1 (2%)	4.45	0.06	0.352	0.28-71.22
	(AA+AG) vs. GG	11 (91.6%)	49 (98%)	0.22	0.76	0.352	0.01-3.59
	(GG+AG) vs. AA	5 (41.6%)	4 (8%)	8.21	0.36	0.010*	1.84-36.57
	A allele	18 (75%)	95 (95%)	0.16	0.80	0.007**	0.04-0.56
	G allele	6 (25%)	5 (5%)	6.33	0.21	0.007**	1.77-22.60
PCOS	AA	52 (65%)	46 (92%)	0.16	0.77	0.000***	0.05-0.49
	AG	24 (30%)	3 (6%)	6.71	0.25	0.001**	1.93-23.42
	GG	4 (5%)	1 (2%)	2.58	0.03	0.360	0.29-23.27
	(AA+AG) vs. GG	76 (95%)	49 (98)	0.39	0.60	0.648	0.04-3.50
	(GG+AG) vs. AA	28 (35%)	4 (8%)	6.19	0.29	0.000***	2.04-18.78
	A allele	128 (80%)	95 (95%)	0.12	0.75	0.001**	0.08-0.56
	G allele	32 (20%)	5 (5%)	4.75	0.15	0.001**	1.79-12.59
TFI	AA	18 (64.28%)	46 (92%)	0.16	0.77	0.007**	0.04-0.55
	AG	8 (28.57%)	3 (6%)	6.27	0.24	0.018*	1.53-25.62
	GG	2 (7.1%)	1 (2%)	3.77	0.05	0.583	0.34-42.21
	(AA+AG) vs. GG	26 (92%)	49 (98%)	0.27	0.72	0.583	0.02-2.97
	(GG+AG) vs. AA	10 (35%)	4 (8%)	6.39	0.3	0.007**	1.80-22.63
	A allele	44 (78.57%)	95 (95%)	0.19	0.76	0.005**	0.06-0.58
	G allele	12 (21.42%)	5 (5%)	5.18	0.17	0.005**	1.73-15.50
UI	AA	10 (71.41%)	46 (92%)	0.22	0.72	0.124	0.05-0.98
	AG	3 (21.41%)	3 (6%)	4.27	0.16	0.226	0.79-23.10
	GG	1 (7.1%)	1 (2%)	3.77	0.05	0.392	0.24-60.26
	(AA+AG) vs. GG	13 (92.8%)	49 (98%)	0.27	0.72	0.392	0.02-4.24
	(GG+AG) vs. AA	4 (28.57%)	4 (8%)	4.60	0.22	0.062	1.02-20.76
	A allele	23 (82.14%)	95 (95%)	0.24	0.72	0.080	0.07-0.89
	G allele	5 (17.85%)	5 (5%)	4.13	0.13	0.080	1.12-15.23

The genotype frequencies of IL-10 between infertile and healthy women were compared by HWE calculation. The differences between the observed and expected values of genotype frequencies were statistically non-significant in Endo, PCOS, TFI, and UI infertility infertile women compared with healthy women ($P > 0.05$), indicating that the distribution of all infertile groups was under Hardy-Weinberg equilibrium (HWE) except the control group in case IL-10 (rs1800872) Table 5.

[DOI: 10.61186/rbmb.12.4.550]

Table 5. HWE test for the genotypes and alleles distributions of IL-10 (rs1800896) (rs1800872) gene in the infertile women and fertile participants.

Case Categories		IL-10 Genotypes (rs1800896) gene					
		AA	AG	GG	HWE p. value	Alleles A	G
Endo	Observed	7 (58.33%)	4 (33.33%)	1 (8.33%)	0.928	18 (75%)	6 (25%)
	Expected	6.75 (56.25%)	4.5 (37.5%)	0.75 (6.25%)		Not estimated	
PCOS	Observed	52 (65%)	24 (30%)	4 (5%)	0.855	128 (80%)	32 (20%)
	Expected	51.2 (54%)	25.6 (32%)	3.2 (4%)		Not estimated	
TFI	Observed	18 (64.28%)	8 (28.57%)	2 (7.14%)	0.725	44 (78.57%)	12 (21. %)
	Expected	17.29 (61.75%)	9.43 (33.67%)	1.29 (4.6%)		Not estimated	
UI	Observed	10 (71.42%)	3 (21.42%)	1 (7.14%)	0.601	23 (164.2%)	5 (35.71%)
	Expected	9.45 (67.5%)	4.11 (29.35%)	0.45 (3.21%)		Not estimated	
Control	Observed	46 (92%)	3 (6%)	1 (2%)	0.033	95 (190%)	5 (10%)
	Expected	45.12 (90.24%)	4.75 (9.5%)	0.13 (0.26%)		Not estimated	

Case Categories		(rs1800872) Gene			HWE p. value	Alleles	
		CC	CA	AA		C	A
Endo	Observed	1 (8.33%)	6 (50%)	5 (41.66%)	0.91	8 (66.66%)	16 (133.33%)
	Expected	1.33 (11%)	5.33 (44.41%)	5.33 (44.41%)		Not estimated	
PCOS	Observed	6 (7.5%)	30 (37.5%)	44 (55%)	0.961	42 (52.5%)	118 (147.5%)
	Expected	5.51 (6.88%)	30.98 (38.72%)	43.51 (54.38%)		Not estimated	
TFI	Observed	2 (7.14%)	11 (39.28%)	15 (53.57%)	0.999	15 (53.57%)	41 (146.42%)
	Expected	2.01 (7.17%)	10.01 (35.75%)	15.01 (53.6%)		Not estimated	
UI	Observed	1 (7.14%)	6 (42.82%)	7 (50%)	0.982	8 (57.14%)	20 (142.85%)
	Expected	1.14 (8.14%)	5.71 (40.78%)	7.14 (51%)		Not estimated	
Control	Observed	2 (4%)	7 (14%)	41 (82%)	0.131	11 (22%)	89 (635.71%)
	Expected	0.6 (1.2%)	9.79 (13.58%)	39.61 (79.22%)		Not estimated	

Evaluation of Serum IL-10 Concentration According to IL-10 (-592C/A, − 1082 A /G) genotypes

The mean IL-10 concentration in the control group is generally higher for the CC genotype and lower for the CA genotype than the AA genotype in the rs1800872 SNP. In all infertile subgroups, the mean IL-10 concentration is generally lower. The highest mean IL-10 concentration was observed in the CC, CA, AA, and AA for Endo, PCOS, TFI, and UI, respectively. In the context of IL-10 (rs1800896), the GG genotype typically exhibits higher concentrations in the control group, while the AG genotype shows lower concentrations. Endo-infertility individuals have lower average IL-10 concentrations, with the AG genotype showing the lowest concentration. In the case of the PCOS group, the GG genotype demonstrates the most elevated mean IL-10 value. In contrast, within the TFI group, the AA genotype exhibits the most significant mean IL-10 level. In contrast, the AG genotype in the UI group displays the maximum mean IL-10 concentration (Fig. 8).

[DOI: 10.61186/rbmb.12.4.550]

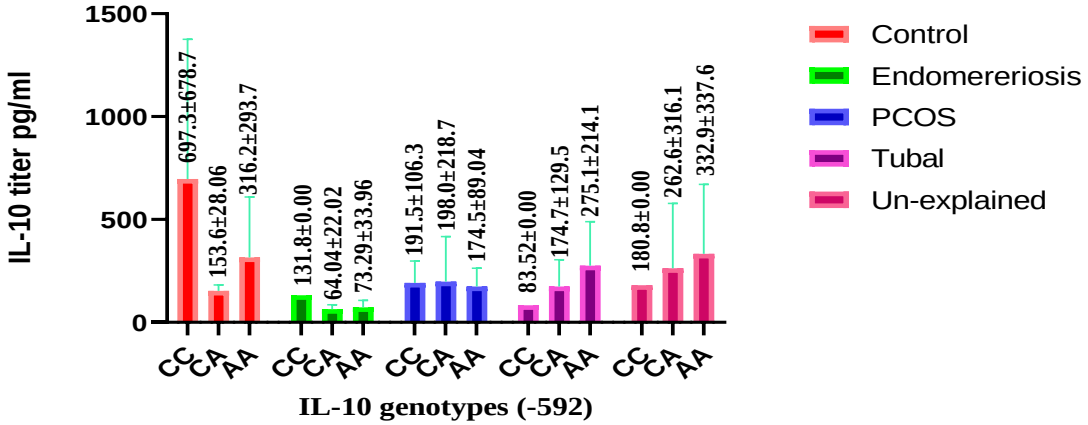

Fig. 7. Mean distribution and standard deviation of serum IL-10 concentration according to IL-10 (-592C/A) genotype among fertile and infertile participants.

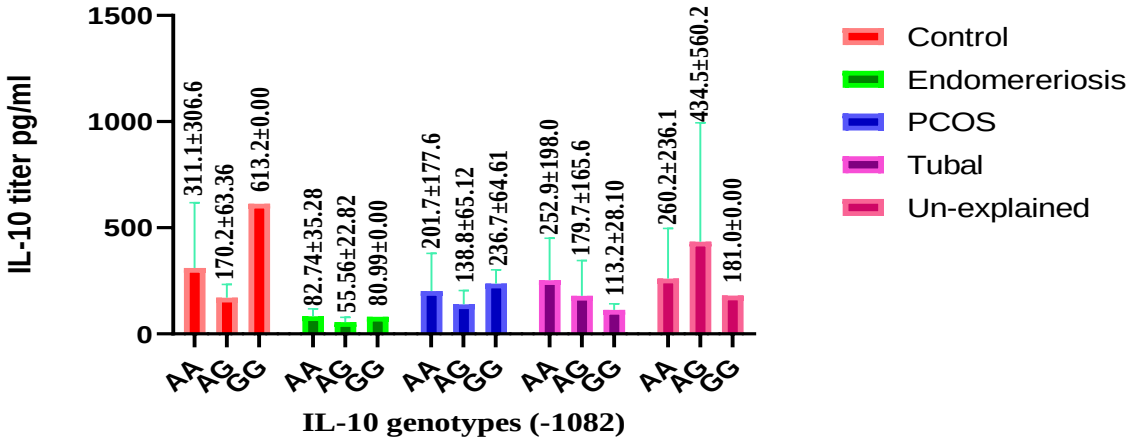

Fig. 8. Mean distribution and standard deviation of serum IL-10 concentration according to -1082 A /G genotypes among fertile and infertile participants

The Relationship between IL-10 Concentration and CT PCR Results

The average IL-10 concentrations remained consistently elevated in the positive *C. trachomatis* group compared to the negative *C. trachomatis* group across all categories except the Endo infertility group (Fig. 9).

The mean IL-10 concentrations in the positive *C. trachomatis* group were as follows: 60.41±19.79 pg/ml in the Endo group, 234.2±210.0 pg/ml in the PCOS group, 250.0±212.9 pg/ml in the TFI group, and 343.4±350.2 pg/ml in the UI group.

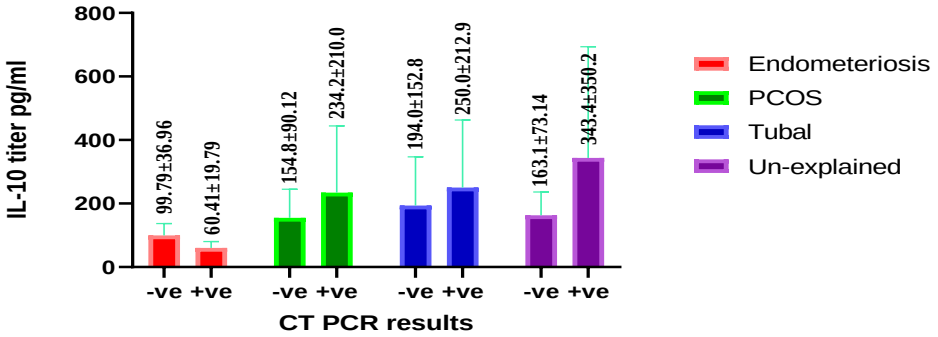

Fig. 9. Mean distribution and standard deviation of serum IL-10 concentration according to *C. trachomatis* PCR results among infertile participants; –ve indicates negative *C. trachomatis* group, +ve indicates negative *C. trachomatis* group.

[DOI: 10.61186/rbmb.12.4.550]

Frequency of the IL-10 genotype in positive C. trachomatis

Our results show that the frequency of the AA genotype is higher than that of the CA and CC genotypes in the case of IL-10 (rs1800872) in PCOS, TFI, and UI, while, in the Endo, the higher risk of *C. trachomatis* infection is CA genotypes. In the case of IL-10 (rs1800896), the frequency of the AA genotype is higher than that of the AG and GG genotypes in PCOS, TFI, and UI, except in the Endo AG genotypes are at higher risk of *C. trachomatis* infection followed by AG genotype (Table 6 and Fig. 10)

Table 6. Frequency of the IL-10 genotype in positive *C. trachomatis.*

IL-10 Genotypes	Genotypes	Infertile Group			
		Endo	PCOS	TFI	UI
-592 C /A	CC	0	2	0	1
	CA	6	7	6	4
	AA	2	21	8	5
-1082 A /G	AA	3	19	11	8
	AG	4	9	2	1
	GG	1	2	1	1

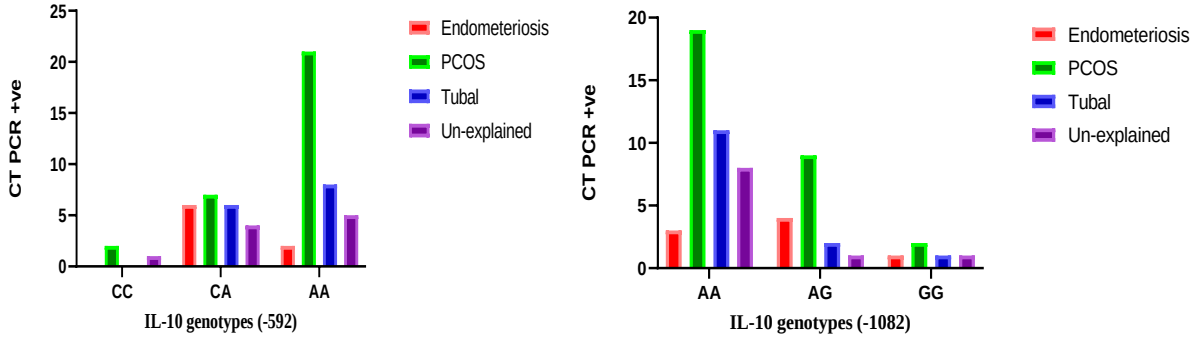

Fig. 10. Estimating serum anti-chlamydial IgM according to IL-10 -592 C/A and − 1082 A /G genotypes among different infertile groups.

Discussion

There is limited data on the prevalence of chlamydial infection, particularly among developing countries such as Iraq, where regular laboratory testing laboratories for this disease are unavailable (14). The age groups participating in the population study were between 20 and 45. Our result aligns with (15); 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 (16).

In our study, all infertile and fertile females were positive for anti-chlamydial IgM antibody using ELISA, but the intensity of response differed between cases. The higher *C. trachomatis* IgM levels suggest a more robust immune response to the infection in the positive *C. trachomatis* group. While the frequency of *C. trachomatis* by PCR technique

[DOI: 10.61186/rbmb.12.4.550]

was 46.26 % in infertile women, in contrast, none of the participants in the fertile group tested positive for the infection. The high prevalence of *C. trachomatis* infection among infertile women highlights the significance of this pathogen as a potential cause of infertility. Our results agree with studies conducted in Iraq; Sahi (17) in Basra revealed that the frequency of infection caused by *C. trachomatis* in women with infertility was 43.2%. The lower prevalence rate of *C. trachomatis* was reported In Iraq, 17.07% by Shamkhi *et al.* (18) and 22% by Ali and Shia (19) in Baghdad; in Egypt, by Abdu Alwadood (20) through using the PCR technique in patients with infertility was 29.3%.

The cause of variances in the rates may be due to the sample size, techniques, lack of medicine, continuous water interruption, and malnutrition that strongly impacted the immune system. Whereas the differences in the rate of *C. trachomatis* using ELISA techniques in serum were higher than in endocervical swab specimens, this may be due to the serum being used, which could show a cross-reactive antibody response with different *Chlamydia* species (21).

In the positive *C. trachomatis* group, the mean *C. trachomatis* IgM levels were higher than the negative *C. trachomatis* group in all infertile groups, which suggests that the presence of *C. trachomatis* infection is linked to increased production of *C. trachomatis* -specific IgM antibodies, indicating an active or recent infection. Permanent tubal destruction and the result of a host immune response to persistent or repeated infection; host genetic variations in the immune response are thought to be contributing to personal variations in illness outcome (22).

The most prevalent cause of infertility was PCOS, which suggests that PCOS has a significant association with infertility in our samples. Salman *et al.* (23) revealed that the causes of infertility vary based on the partner's age and the marriage's age, and PCOS is still the most prevalent cause of tubal factor infertility.

The group with UI had the most significant percentage of positive *C. trachomatis* PCR findings, followed by those with Endo, TFI, and PCOS. Chlamydial infection may impact fertility by creating anti-sperm antibodies, impeding sperm motility and egg contact, and perhaps causing antigen overlap (Abdella *et al.*, (24). Endometriosis inflammation can harm sperm or eggs by interfering with their passage across the fallopian tubes and uterus. Adhesions or scar tissue may obstruct the fallopian tubes in severe cases of endometriosis. Cervicitis, urethritis, or vaginitis are the most common symptoms of *C. trachomatis* genital infections; however, some infections are asymptomatic. Endometriosis can develop from untreated infections that ascend the female vaginal system (25).

Chlamydia trachomatis infection increases IL-10 levels, indicating immune response and activation of anti-inflammatory mechanisms. Our results align with Wang *et al.* (26), who revealed that the mean IL-10 concentration in cervical secretion was higher in positive chlamydial infection than in the negative Chlamydial sample. It has been noted that *C. trachomatis* stimulated cervical cells secreted higher levels of IL-1, IL-6, IL-8, and IL-10 in *C. trachomatis*-positive infertile women (27, 28). In contrast, Okpalaji *et al.* (29) showed no significant increase in their level of IL-10 in the infertile women compared with the fertile women.

In IL-10 -592, the CA and CC genotypes have a higher risk for infertility than women with the AA genotype. The C allele is associated with an increased risk for infertility, except in PCOS, which is an A allele. In the case of IL-10 -1082, the AG genotype has a greater risk with the G allele. Jukema *et al.* (5) revealed that *C. trachomatis*-positive women with IL10 − 1082 GG have a threefold higher odds ratio for developing late complications. Öhman *et al.* (22) and Kinnunen *et al.* (30) showed that the AA genotype and A allele in IL-10 − 1082 SNP significantly increase disease severity and the probability of severe tubal damage in women with *C. trachomatis*-related infertility. Reduction in IL10

[DOI: 10.61186/rbmb.12.4.550]

production was linked to pregnancy loss and increased pre-eclampsia; IL10 polymorphisms at locations 2849, 1082, and 592 were associated with miscarriages (31). A significant difference in genotype distributions for cases in different populations may be due to the IL-10 − 1082 SNP's unique distribution in Iraq.

Chlamydia trachomatis impacts gene expression, epithelial disturbances, and micro-abrasions, causing inflammation, immunological mediators, and disease development (32, 33). Further research is needed to understand the role of IL-10 genotypes in *C. trachomatis* infection susceptibility, clinical outcomes, and disease incidence.

In conclusion, IL-10 gene polymorphisms may contribute to *C. trachomatis*-associated infertility in Kurdish women, with higher *C. trachomatis* IgM levels in positive groups. Endocervical swabs outperformed ELISA approaches in detecting *C. trachomatis*, and ELISA showed low sensitivity.

Acknowledgment
All the volunteers appreciate the authors. In addition, the Biotechnology Laboratory of the Biology Department, College of Education, Salahaddin University, assisted in executing all laboratory studies.

Conflict of interest
The authors claim to have no conflicts of interest.

Funding
There was no financial assistance.

Ethical Approval
The Biology Department, College of Education, Salahaddin University's Scientific Committee authorized this study.

References
1. Mortazavi SM, Tarinjoo A, Dastani S, Niyazpour M, Dahaghin S, Mirnejad R. Molecular Detection of Sexually Transmitted Infections in Women with and without Human Papillomaviruses Infection Who Referred to Tehran West Hospitals in Iran. Rep Biochem Mol Biol. 2021;10(3):387-395.
2. Price MJ, Ades AE, Welton NJ, Simms I, Macleod J, Horner PJ. Proportion of Pelvic Inflammatory Disease Cases Caused by Chlamydia trachomatis: Consistent Picture From Different Methods. J Infect Dis. 2016;214(4):617-24.
3. Report on global sexually transmitted infection surveillance, 2018. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.
4. Ohman H, Tiitinen A, Halttunen M, Paavonen J, Surcel HM. Cytokine gene polymorphism and Chlamydia trachomatis-specific immune responses. Hum Immunol. 2011;72(3):278-82.
5. Jukema JB, Hoenderboom BM, van Benthem BHB, van der Sande MAB, de Vries HJC, Hoebe CJPA, et al. Can Previous Associations of Single Nucleotide Polymorphisms in the TLR2, NOD1, CXCR5, and IL10 Genes in the Susceptibility to and Severity of Chlamydia trachomatis Infections Be Confirmed? Pathogens. 2021;10(1):48.
6. Broeze KA, Opmeer BC, Coppus SF, Van Geloven N, Den Hartog JE, Land JA, et al. Integration of patient characteristics and the results of Chlamydia antibody testing and hysterosalpingography in the diagnosis of tubal pathology: an individual patient data meta-analysis. Hum Reprod. 2012;27(10):2979-90.
7. Datta A, Tuz Zahora F, Abdul Aziz M, Sarowar Uddin M, Ferdous M, Shalahuddin Millat M, et al. Association study of IL10 gene polymorphisms (rs1800872 and rs1800896) with cervical cancer in the Bangladeshi women. Int Immunopharmacol. 2020;89(Pt B):107091.

[DOI: 10.61186/rbmb.12.4.550]

8. Williams AE, Edwards L, Humphreys IR, Snelgrove R, Rae A, Rappuoli R, Hussell T. Innate imprinting by the modified heat-labile toxin of Escherichia coli (LTK63) provides generic protection against lung infectious disease. J Immunol. 2004;173(12):7435-43.
9. Moumad K, Khaali W, Benider A, Ayoub W, Cherif M, Boualga K, et al. The Involvement of Interleukin-10 Promoter Genetic Polymorphism in Epstein-Barr Virus-Associated Nasopharyngeal Carcinoma from North Africa. Eurasian J Med Oncol. 2022; 6(3):232-240.
10. Darville T, O'Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L, Ojcius DM. Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. J Immunol. 2003;171(11):6187-97.
11. Xiang W, Yu N, Lei A, Li X, Tan S, Huang L, Zhou Z. Insights into Host Cell Cytokines in Chlamydia Infection. Front Immunol. 2021;12:639834.
12. Wei M, Chen D, Feng G, Mao X, Wu L, Chai W, Zhang J. Association of infertility cause with perinatal outcomes in a freeze-all policy: an analysis including 10,151 singleton newborns. AJOG Glob Rep. 2022;3(1):100098.
13. Mohammed MA, Omer AF. Molecular detection of Chlamydia trachomatis among gynecological patients attending Khartoum Teaching Hospital. J Bacteriolo Res. 2012;4(4):42-5.
14. Gahanbarzade N, Ramazani E, Yousefi M, Zardast M, Zare-Bidaki M. Prevalence of Endocervical Chlamydia
trachomatis Infection and Related Risk Factors Among Women Attending Gynecology Clinic of Birjand University of Medical Sciences, East of Iran. Arch Clin Infect Dis. 2021;16(2):e110647.
15. Ajani TA, Fayemiwo SA, Oluwasola TAO, Anaedobe CG, Ajani MA, Bakare RA. Prevalence of Asymptomatic genital Chlamydia trachomatis infection among infertile women in Ibadan, Nigeria using Polymerase Chain Reaction. Indian J Med Research and Pharmaceutical Sciences. 2017;4(9):13-23.
16. Mawak JD, Dashe N, Agabi YA, Panshak BW. Prevalence of genital Chlamydia trachomatis infection among Gynaecologic clinic attendees in Jos, Nigeria. Shirez E Med Jl. 2011;12(2):100–106.
17. Sahi AA, Mohammed KA. Comparison of immunological and molecular methods for the detection of Chlamydia trachomatis among infertile women in Basra, Iraq. Ann Trop Med Public Health. 2020;23:232–7.
18. Shamkhi GJ, Alkhuzai RAH, Al-Shukr NMK. Molecular Genotyping of Chlamydia trachomatis in Iraqi Married Pregnant and Non-Pregnant Women. Arch Razi Inst. 2022;77(2):761-769.
19. Ali, MK, & Shia, JS. Detection of Chlamydia trachomatis and Neisseria gonorrhoeae in genitourinary specimens in Iraq women by real time PCR assay. Res J Pharm Biol Chem Sci, 2018;9(6), 799–808.
20. Abdu Alwadood, M. Prevalence rate of chlamydia trachomatis infection in Egyptian infertile women. Al-Azhar J Pharma Sci, 2013;48(2):1-15.
21. Salman YJ, Ahmed ES, Taqi RH. Efficacy of some laboratory samples and techniques in detecting Chlamydia trachomatis infection among women in Kirkuk Province. Int J Curr Res Aca Rev. 2018;6(10):39-50.
22. Ohman H, Tiitinen A, Halttunen M, Lehtinen M, Paavonen J, Surcel HM. Cytokine polymorphisms and severity of tubal damage in women with Chlamydia-associated infertility. J Infect Dis. 2009;199(9):1353-9.
23. Salman ST, Khalaf SK, Hussain AA. Infertility rate and relationship between infertility status and microbial infections among women in Baquba city. City.2022;6(S2):1175-86.

[DOI: 10.61186/rbmb.12.4.550]

24. Abdella RM, Abdelmoaty HI, Elsherif RH, Sayed AM, Sherif NA, Gouda HM, et al. Screening for Chlamydia trachomatis in Egyptian women with unexplained infertility, comparing real-time PCR techniques to standard serology tests: case control study. BMC Womens Health. 2015;15:45.
25. Lin W-C, Chang CY-Y, Hsu Y-A, Chiang J-H, Wan L. Increased risk of endometriosis in patients with lower genital tract infection: a nationwide cohort study. Medicine. 2016;95(10) :e2773.
26. Wang C, Tang J, Geisler WM, Crowley-Nowick PA, Wilson CM, Kaslow RA. Human leukocyte antigen and cytokine gene variants as predictors of recurrent Chlamydia trachomatis infection in high-risk adolescents. The Journal of infectious diseases. 2005;191(7):1084-92.
27. Rodgers AK, Wang J, Zhang Y, Holden A, Berryhill B, Budrys NM, et al. Association of tubal factor infertility with elevated antibodies to Chlamydia trachomatis caseinolytic protease P. Am J Obstet Gynecol. 2010 Nov;203(5):494.e7-494.e14.
28. Mascellino MT, Boccia P, Oliva A. Immunopathogenesis in Chlamydia trachomatis Infected Women. ISRN Obstet Gynecol. 2011;2011:436936.
29. Okpalaji C, Okerengwo A, Okpani A, Chinko B, Bamigbowu E. Serum cytokine concentrations in infertile and fertile women. A preliminary study in port Harcourt, Nigeria. IOSR J Dent Med Sci 2016; 15(9): 77–9.
30. Kinnunen AH, Surcel HM, Lehtinen M, Karhukorpi J, Tiitinen A, Halttunen M, et al. HLA DQ alleles and interleukin-10 polymorphism associated with Chlamydia trachomatis-related tubal factor infertility: a case–control study. Hum Reprod. 2002;17(8):2073-8.
31. van Dunné FM, de Craen AJ, Helmerhorst FM, Huizinga TW, Westendorp RG. Interleukin-10 promoter polymorphisms in male and female fertility and fecundity. Genes Immun. 2006;7(8):688-92.
32. Miraghaee SS, Sohrabi M, Jalili C, Bahrehmand F. Assessment of GSTO1 (A140D) and GSTO2 (N142D) Gene Polymorphisms in Iranian Women with Polycystic Ovarian Syndrome. Rep Biochem Mol Biol. 2020;9(1):8-13.
33. Arcia Franchini AP, Iskander B, Anwer F, Oliveri F, Fotios K, Panday P, Hamid P. The Role of Chlamydia Trachomatis in the Pathogenesis of Cervical Cancer. Cureus. 2022;14(1):e21331.

[DOI: 10.61186/rbmb.12.4.550]