THE RELATIONSHIP BETWEEN SINGLE NUCLEOTIDE POLYMORPHISM OF INTERLEUKIN -10 GENE PROMOTER (-1082 A/G) WITH INFECTION CHLAMYDIA TRACHOMATIS INFERTILE IRAQI WOMEN.

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ABSTRACT

Aim of this studty to identify the risk of single nucleotide polymorphisms (SNPs) of IL-10 promoter variants -1082A/G associated with *Chlamydia trachomatis* and tubal factor infertility. Out of 100 women were attending different hospitals in Baghdad included the Gynaecology Departments of Women Health Center at Al-Elwyia Obstetrics Hospital, Ibn Al balady Maternity and Children's Hospital , Kamal al-Samarrai hospital Fertility Center infertility treatment and In Vitro Fertilization (IVF) (20 control and 80 women with infertility) with an age range of 16-40 years. DNA was extracted from the endocervical swabs of all infertili women, to investigate the bacteria using Real time -PCR technique and venous blood sample (2 ml) was obtained for DNA detection of IL-10Single Nucleotide Polymorphisms. The results revealed the Diagnosis of *Chlamydia trachomatis* by Real time-PCR was significant responsible with infection in infertile Iraqi women. (OR= 0.733; 13.75%, P= 0.041). Among 80 infertilie women and patients women with infertility diagnosis of *Chlamydia trachomatis* were examined IL-10(-1082) promoter gene polymorphisms were not found to be associated with *Chlamydia* infections, indicating that this gene may not be involved in Chlamydia pathogenesis in Iraqi infertile women But in this study the two new mutation showed (SNP -1218,-1213) was detected in the promoter region of the Interleukin-10 gene in infertile Iraqi women (AATT…..ATT),(GGC….GAC) mutations in 11 women with Infertilty associated with *Chlamydia trachomatis* infection

*Keywords: *Chlamydia trachomatis*, female infertility, Real time -PCR, endocervical swabs,Blood, , Sequencing, Interleukin -10 Gene Promoter (-1082 A/G).
*Part of ph.D.Dissertation of the first author

العلاقة بين التعدد الشكلي لجين الانترلوكين -10في منطقة المحفز (-1082 A/G) والاصابة *Chlamydia trachomatis* في النساء العراقيات العقيمات

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المستخلص

الهدف من الدراسة تحديد مخاطر التعدد الشكلي SNP لمنطقة المحفز لجين الانترلوكين -10 (-1082A/G) المرتبطة بالكلاميديا والعقم الانبوبي . تم فحص 100 امرأة من المراجعات لمستشفيات مختلفة في بغداد تضمنت قسم الامراض النسائية لمركز صحة المرأة في مستشفى العلوية للولادة ومستشفى كمال السامرائي مركز الخصوبة وعلاج العقم والتخصيب الخارجي ومستشفى ابن بلدي للاطفال والنسائية وشملت (80 امراة عقيمة و20 سيطرة)تتراوح اعمارهم 16–40 سنة تم استخلاص الحامض النووي من مسحات عنق الرحم وذلك للتحري عن لبكتريا *Chlamydia trachomatis*باستخدام تقنية Real time PCR .في حين استخلاص الحامض النووي من الدم لغرض تحديد التعدد الشكلي لجين الانترلوكين في منطقة المحفز.اظهرت النتائج بان هنالك احد عشر عينة موجبة من ضمن 80 عينة التي تم اختبارها باستخدام تقنيةReal –time pcr والتي اعطت فروقات معنوية مرتبطة مع النساء العقيمات ,(OR= 0.733; 13.75%, P= 0.041). في حين لم يظهر اي ارتباط معنوي في منظقة الممهدد لجين الانترلوكين -10 (-1082A/G) في النساء العقيمات مما يشير على انه هذاة الجين ليس له علاقة للاصابة بالكلاميديا في النساء العراقيات قي خين حددت طفرتين جديدة(-1213,-1218 SNP) في موقعين جددين (AATT…..ATT),(GGC….GAC) في 11 امراة مصابة بكلاميديا.

*الكلمات المفتاحية:الكلاميديا التراخوماتية، نساء عقيمات. تقنية Real time –PCR، مسحات عنق الرحم، دم، تسلسلات النيوكليواتيدية، محفز لجين الانترلوكين -10.(-1082 A/G) *جزء من اطروحة دكتوراة للباحث الاول

*Received:19/5/2017, Accepted:28/12/2017

INTRODUCTION

Interleukin-10 (IL-10) has potent pleiotropic effects in immune regulation and inflammation. IL-10 is expressed in T cells, macrophages, monocytes, dendritic cells, mast cells, B cells, eosinophils, keratinocytes, epithelial cells, and a number of tumor cell lines (13). The main functions of IL-10 include restricting and ending inflammatory response, preventing pro-inflammatory cytokine secretion, and regulating the differentiation and proliferation of immune cells (T cells, B cells, natural killer cells and mast cells) (27). It is an important cytokine because it is its necessaries for immune response it be controlled an excessive response which may lead to damage the host and can also lead to a number of conditions (15; 27). The gene encoding human IL-10 is located on chromosome 1q31-32. It covers a total of 5.1 kb pairs and comprises five exons and four introns as showed in figure (2-4). The IL-10 gene encodes a 178 amino acids long protein, which is secreted after cleavage of the 18 amino acids comprising signal peptide. The IL-10 promoter is highly polymorphic containing, two microsatellites and three single nucleotide polymorphisms (SNPs) (5).

Promoter region polymorphisms appear to be correlated with variations in transcription. Three of several polymorphic sites within the promoter region of IL-10 have been described in some detail. These are -1082 A to G substitution (rs1800896) where A is the ancestral allele , -592 C to A substitution (rs1800872) where C is the wild-type allele, and -819 C to T substitution (rs1800871) where C is the common allele according to the National Center for Biotechnology Information (NCBI) SNP database (2; 14). These SNPs are in linkage disequilibrium and form three haplotypes: GCC, ACC and ATA, where GCC is usually associated with high IL-10 production and ACC and ATA with low production (25). The functional polymorphisms in selected cytokine genes including IL-10, interferon gamma (IFN-Y), Tumour necrosis factor alpha (TNF-α) revealed an increase in severe tubal damage in women with infertility caused by *Chlamydia* when certain IL-10 and TNF-α alleles were present (18). In terms of cytokine secretions in *Chlamydia trachomatis* -positive infertile women, it has been reported that *Chlamydia trachomatis* stimulated cervical cells secreted significantly higher levels of IL-1β, IL-6, IL-8 and IL-10. This indicated that the cytokine secretion profile of cervical cells may produce vital information to indicate the outcome (i.e. fertile or infertile) of a chlamydial infection of the female genital tract (3). Others (9) have reported that IL-1β, IL-4, IL-5 and IL-6 as well as IL-10 levels were found to be higher in *Chlamydia trachomatis* membrane protein (Inc protein)-stimulated cervical cells of *C. trachomatis*-positive infertile women compared to fertile women infected with *Chlamydia* . recently a unique link between elevated levels of anti-Chlamydial caseinolytic protease P (ClpP) and tubal factor infertility was identified in 21 tubal factor infertility patients (19). Host genetic factors are known to modulate the immune defence mechanisms to a *Chlamydia* is infection thus determining the occurrence of *Chlamydia*-induced tubal factor infertility.

MATERIAL AND METHODS

Specimens collection

Endocervical swab samples were collected from 100 women (80 samples from Infertile women and 20 samples from control) . For each patients two endocervical swabs by speculum examination were obtained from all patients by the gynecologist. Then placed in the 300µl phosphate buffered saline (Ph 2-4) and extraction direct by DNA extraction kit (AmpliSens® \Russia) according to the manufacturer's directive DNA was stored at -20°C until used.

DNA extraction from blood

Total genomic DNA isolated from the whole frozen blood depending on the manufacturer's directive using the Genomic DNA Extraction kit geneaid /UK.DNA Isolation kit

Concentration and purity of DNA

The determination of DNA quality and concentration in samples were performed by both spectrophotometric analysis and running on 0.8% agarose gels. Optical density ratios from spectrophotometric analysis were evaluated and only good-quality DNA samples were used in PCR (20).

Diagnosis of *Chlamydia trachomatis* by Real time- PCR: *Chlamydia trachomatis*-

FRTPCR kit (AmpliSens® \ Russia) is a qualitative test that contains the Internal Control (Internal Control-FL (IC)). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition**.** PCR Reaction was performed in a 25 µl total volume for sample also performed for positive control (C+), negative control (C–) and Internal Control (IC) according to the manufacturer's directive consisting of 5 (µl) PCR-mix-1-FL *C. trachomatis*, 10 µl PCR-mix-2-FL, 10 µl Template DNA.

PCR Amplification profiles and Data analysis: The reaction conditions of Real-time PCR for **Diagnosis of *C. trachomatis*** were 15 min hot-start activation at 95°C on 1 cycle, (15sec denaturation at 95°C and 20 sec annealing at 60°C and 15sc at 72°C extension on 5 cycles) and(5sec denaturation at 95°C and 20 sec annealing at 60°C (*Fluorescent signal is detected in the channels for the FAM and JOE fluorophores) on 40 cycles 15sc final extension at 72°C) Analysis of results was performed by the Rotor-Gene Q Software 2.3.1.49 of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels: The signal of the *Chlamydia trachomatis* DNA amplification product is detected in the channel for the FAM fluorophore. The signal of the IC DNA amplification product is detected in the channel for the JOE fluorophore.

Detection Single Nucleotide Polymorphism of *IL* -10 Gene Promoter (-1082 A/G): Polymorphisms in the promoter region of IL-10 were identified using the National Center for Biotechnology Information (NCBI) SNP database. The particular IL-10 SNPs included in this study were as follows: SNP change A / G for –1082, NCBI SNP cluster ID rs1800896. The next experiment Detection The IL-10 promoter gene polymorphism of a single nucleotide at position -1082 (A/G) was investigated by using conventional pcr technique.

Table 1. Sequence of primers utilized for detection Single Nucleotide Polymorphism of IL -10 Gene Promoter (-1082 A/G)

Primers	Sequences 5´→ 3´	Band size/bp	Reverence
Forword (F) Reverse (R)	CAAGACAACA CTACTAAGGC ATTGGCCTTAG AGTTTCTTTTA G	192 bp	10

PCR Reaction was performed in a 20 µl total volume, were contain10 µl PCR Mastermix(Gobiz/ Korea) , 2µl Primer forward , 2µl Primer reverse, 4µl Template DNA, 2µl RNase –free water. The reactions were carried out in a thermo cycler(BioRad-Germany) with the following profile: were initial 10 min denaturation at 94°C on 1 cycle, (15sc denaturation at 94°C, 15sec annealing at 60°C and 1min elongation at 72°C on 35 cycles) and 7min final extension at 72°C on 1 cycle.

PCR Products Sequencing

The PCR products (20infertile group and 20 for control group) of the analyzed (IL-10) Promoter gene and primers were sending to Macrogen Company (U.S.A) for sequencing and The sequences were analyzed using BioEdit software, version 7.0.1 and BLAST basic alignment tool (www.ncbi.nlm.nih.gov).

RESULTS AND DISCUSSION

Diagnosis Of *Chlamydia Trachomatis* By Real Time-PCR: The result of the qPCR revealed significant association with infertile women (OR= 0.733; 13.75%, P= 0.041), among 80 infertili women as showed in table. 2 A significant association between infertility and increased prevalence of *C. trachomatis* infection is shown in this study and agrees with (21;1)

Table 2. Diagnosis of *Chlamydia trachomatis in* women infertility using Qpcr

Sample	IC	NCA	C+	C–	Ct	%
Patients /80	+	-	+	-	**11**	**13.75**
Controls /20	+	-	+	-	**0**	**0.00**
P-value	--	--	--	--	--	**0.041***
O.R.						**0.733**

IC= Internal Control, NCA= Negative Control of Amplification, C+= Positive Control of Amplification, C– = Negative Control of Extraction, Ct= *Chlamydiatrachomatisin*.OR= **Odds Ratio** * (P<0.05).

The amplified product is detected with the use of fluorescent dyes. These dyes are linked to

oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The Internal Control (IC). It must be used in the extraction procedure in order to control the extraction process of each individual sample and to identify possible reaction inhibition (6). In this study the samples were tested by relative quantitative depending on the "housekeeping gene" used as a control for experimental variability (28) and quantification of copy number were accomplished using the standard curve method depending on threshold cycle (Ct) of houskeeping gene Amplification was deemed efficient for a sample if the conforming Internal control demonstrated the same (Ct) (±1) of positive control. A sample was considered negative if the signal did not increase within 35 cycles. An amplification plot shown in Figure(1- A , B)

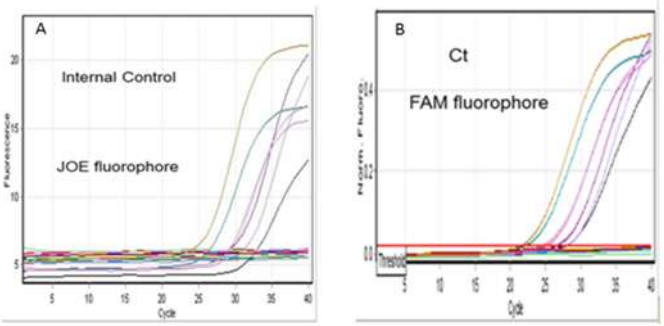

Fig-1: A q-PCR amplification plot (fluorence vs Cycle) A: The signal of the Internal Control (IC) is detected in the JOE fluorophore. B: The signal of the Chlamydia trachomatis DNA amplification product is detected the FAM fluorophore

The amplification was registered as a Ct value. The lower Ct value indicated the presence of higher copies of the target and vice versa. In terms of gene expression, high Ct values indicated low gene expression and low Ct value indicates a high gene expression (12;16). The range of Ct value for Diagnosis of *Chlamydia trachomatis* in women infertility patients was between 21.41-31.03 with a mean ± SD(25.88 ± 4.12). In this study the most frequent cause of infertility was blocked fallopian tube in 6 from 11 patients (54.5%) of women, followed by the presence of blocked fallopian tubes and removed another in 5 (45.5%) patients as showed in Figure.2 . However, it was found association between detection of *C. trachomatis* in infertile women and the causes of infertility. (Table-3) Signs and symptoms could be identified as significantly associated with *C. trachomatis* infection in infertile women. this agreed with the study by (8) who was detected *C. Trachomatis* by PCR in the endocervix of 4 out of 150 cases (2.67%), two of them belong to infertile Egyptian women with tubal blockage. So, repeated chlamydial infections are a risk factor for Pelvic Inflammatory Disease (PID). and more importantly is linked to inflammation of fallopian tubes, which can result in scarring and thus chronic pelvic pain, infertility and ectopic pregnancy (24). Over 98% of all ectopic pregnancies, are located in the fallopian tubes. Previous damage of the fallopian tubes by infection with *C. trachomatis* belong to the major risk agent of ectopic pregnancy and tubal factor infertility (23;22)

Table 3. signs and symptoms could be identified as significantly associated with *C. trachomatis* infection in infertile women

No. of patients sample	Symptoms and signs	%
6	blocked fallopian	54.5
5	blocked fallopian tubes and removed another	45.5
Total \ 11		100%

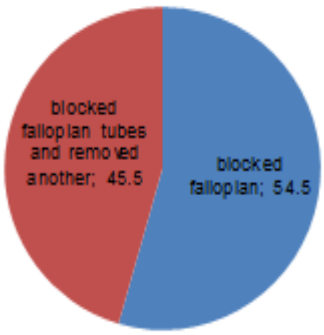

Fig 2. Percentage of frequent cause of infertility was blocked fallopian tubes and removed another in 11 Patients.

The current study showed that the bilateral tubal obstruction was similar in primary and secondary infertility groups and this agrees with (4) that also stated similar bilateral tubal obstruction in primary and secondary infertility groups from Oman, in addition to that, previous pelvic surgery might be the cause of the tubal obstruction in the secondary infertility group.

Amplification of target gene: (The *IL10* promoter gene polymorphism of a single nucleotide at position -1082 : In this stage, the twenty isolates from infertile women (elevene are diagnosis infection of *Chlamydia trachomatis*) and twenty isolates from healthy women were used to amplify in the promoter region of IL-10 at position -1082 on the genome. Polymerase chain reaction was done under optimal amplification conditions by using specific primers .Results a in Figure .3 showed that the amplified products were appeared as clear bands after electrophoresis on agarose gel (2%) with molecular size of 192 base pair in presence of 10000 bp ladder marker. This result agree with (10) who are proved the size of this fragment is 192 bp .These fragments represent the region of ID rs1800896 within the promoter region of IL-10 which located on the long arm of chromosome 1(1q31-32) and this is consistent with the research of (25).

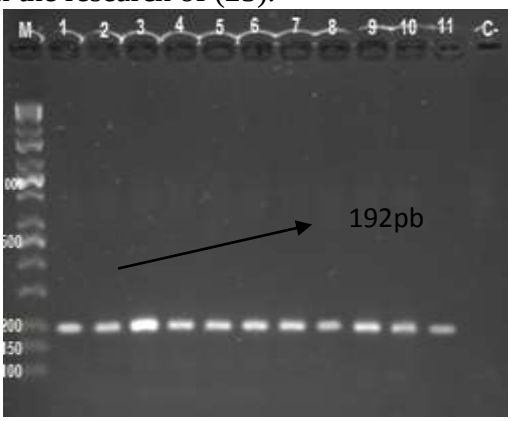

Fig 3. Gel electrophoresis for PCR products of *IL-10* -1082 on agarose gel (2%) after electrophoresis for 1hour at 5v/cm^{2}in the presence of 10000 bp(M: DNA Ladder marker; Lane (1)-(11): patients band;C: healthy control)

(IL-10 Promoter) gene Sequence analysis and screening the adjacent region: A twenty infertile Women (including eleven patients diagnosed with *Chlamydia trachomatis* infection) were examined for IL-10(-1082) promoter gene polymorphisms were found not to be associated with Chlamydia *trachomatis* infections, indicating that this gene may not be involved in Chlamydia *trachomatis* pathogenesis in Iraqi infertile women. This results is in agreement with (7) who showed not significant -1082,- 819,- 592 Haplotype associated with Tubal infertility in 70 Kenyan women . While not agreement with several genetic association studies related to *C. trachomatis* genital diseases have been published . Candidate gene approaches have been used to study the genetic risk factors of susceptibility to and severity of *C. trachomatis* diseases. *IL10* gene has been associated with disease. As regards STIs, the IL10 -1082/- 819/-592 GCC haplotype is associated with a lower risk of recurrent infection (26) and the IL10 -1082 AA genotype has been found to be more common among TFI cases (37%) than in controls (25%). Together with HLADQB1* 0602 the IL10 -1082 AA genotype has been associated with TFI ($p = 0.005$) (11). (18) showed that IL-10 -1082 AA genotype and the TNF-alpha -308 A allele increase the risk of severe tubal damage in women with infertility associated with *C. trachomatis* in a group of 114 Finland women. Furthermore, in another search for the same researchers, they noted a significant relation in the respone of lymphocyte proliferation (LP) to all chlamydial antigens and the IL-10 -1082 SNP (p0.03) but not to the IL-10 -819/-592 SNPs (17). But in this study the two new mutation showed (SNP -1218,-1213) was detected mutant site in the promoter gene region of the Interleukin-10 gene in infertile Iraqi women(AAT…..ATT), (GGC….GAC) there were mutations in 11 women with Infertilty as showed in figure (4-a, 4-b).

Fig 4. Alignment of IL-10 Promoter gene in region -1082 was not detected any mutant site in infertile Iraqi women using automated sequencer was analyzed by Blast data

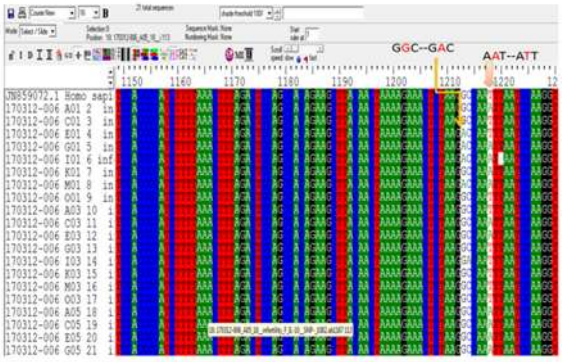

Fig 4b. Alignment of *IL-10* Promoter gene in region (SNP-1218,-1213) was detected mutant site(AAT…..ATT),(GGC….GAC) in infertile Iraqi women using automated sequencer was analyzed by Blast data

The study found a single mutation in some patients 1(5%) mutant site in the promoter gene region(-1213) ;7(35%) mutant site in the promoter gene region(-1218) and also are found two mutation in other patients in the promoter gene region(-1213- -1218) 3(15%) (Table - 4).

Table 4. Analysis of (IL-10 Promoter) gene in Infertility Women

No. of patients	-1082		-1213		-1218		-1213/-1218	Total
	Wilde type GAA	Mutant type GAA	Wilde type GGC	Mutant type GAC	Wilde type AAT	Mutant type ATT	GGC ➔GAC AAT ➔ATT	
20	0(0%)		1(5%)		7(35%)		3(15%)	11(55%)

with blocked fallopian mutant site in the promoter gene region (-1218) While 5(45%) blocked fallopian tubes and removed another Distributed 3 (27.3) two mutant site in the promoter gene region(-1218; -1218) and 1(9.1) one mutant site in the promoter gene region(-1218 ; -1218) Respectively.

Table 5. signs and symptoms could be identified as significantly associated with *C. trachomatis* infection with detected mutant site in the promoter gene region of the IL-10 gene in infertile Iraqi

No. of patients	Symptoms and signs	%
-1218 (6) -1218 -1213 3 (27.3) -1213 1(9.1) -1218 1(9.1)	blocked fallopian blocked fallopian tubes and removed another	54.5% 45.5%
11		100%

Genotypes and Allele Frequencies for IL10 gene polymorphsim -1213; -1218 polymorphism: Distribution of the observed -1213 G/A genotype and allele frequencies in the control and infertility patients groups are shown in (Table -6). The highest genotype in the control group was G/G (95.0 %) followed by G/A genotype (5.0 %) and 0 % for A/A genotype. In whole infertile patients, the highest genotype was the G/G (80.0 %) followed by G/A (15.0 %) and A/A genotypes (5.0%). The odds ratio for the -1213 G/A was 3.5 (0.3-37.6) (P=0.29) the difference from the control was statistically non-significant, p>0.05. As well as, the -1213 AA revealed a statistically significant with the control an odds ratio was 3.54 (0.1-93.0) (P=0.03) .The wild type GG genotype and wild type G allele were taken as reference. For -1213 A allele, the O.R was 5.5 (0.6-50.0) P=0.12 with no significant difference from the control0.

Table 6. Comparison of the Genotype and Allele of IL10 gene polymorphsim -1213 between Patient group and Healthy group

IL10 gene polymorphsim -1213	Frequencies (%)		P value	Odd ratio (95% CI)
	Healthy (n=20)	Patient (n=20)		
GG	95.0 (n=19)	80.0 (n=16)	---	1.00 (Reference)
GA	5.0 (n=1)	15.0 (n=3)	0.29	3.5 (0.3-37.6)
AA	0.0 (n=0)	5.0 (n=1)	0.03	3.54 (0.1-93.0)
G	95.0 (39)	75.0 (35)	---	1.00 (Reference)
A	5.0 (1)	25.0 (5)	0.12	5.5 (0.6-50.0)

Distribution of the observed For -1218 A/T genotype and allele frequencies in the control and infertility patients groups are shown in (Table -7). The highest genotype in the control group was A/A (85.0 %) followed by A/T genotype (15.0 %) and 0 % for T/T genotype. In whole patients, the highest genotype was the A/A (50.0 %) followed by A/T (35.0 %) and T/T genotypes (15.0%). The odds ratio for -1218 A/T was 5.95 (1.0-34.4) (P= 0.046) the similar the control was statistically significant, p<0.05. As well as, the -1213 TT revealed a statistically non-significant with the control

with an odds ratio was 5.1 (0.4-55.8) (P=0.34) .the wild type AA genotype and wild type A allele were taken as reference. For -1218 T allele, the O.R was 4.3 (1.2-14.7)P=0.019 with significant with the control.

Table 7. Comparison of the Genotype and Allele of IL10 gene polymorphsim -1218 between Patient group and Healthy group

IL10 gene polymorphsim -1218	Frequencies (%)		P value	Odd ratio (95% CI)
	Healthy (n=20)	Patient (n=20)		
AA	85.0 (n=17)	50.0 (n=10)	---	1.00 (Reference)
AT	15.0 (n=2)	35.0 (n=7)	0.046	5.95 (1.0-34.4)
TT	0.0 (n=1)	15.0 (n=3)	0.34	5.1 (0.4-55.8)
A	80.0 (36)	75.0 (27)	---	1.00 (Reference)
T	20.0 (4)	25.0 (13)	0.019	4.3 (1.2-14.7)

Distribution of the observed For combination of SNPs -1213 (GGC- GAC) and -1213 (AAT - ATT) genotype and allele frequencies in the control and infertility patients groups are shown in (Table -8) The highest genotype in the control group was GG/AA (85.0 %) followed by GG/AT, GA/AAand GA/AT genotype (5.0 %) and 0 % for GG/TT and AA/TT genotype. In whole patients, the highest genotype was the GG/AA (45.0% %) followed by GG/AT (25.0%%), GA/AA genotypes (20.0%) and (5.0 %) GA/AT, AA/TT The odds ratios were calculated by comparison of control individuals and infertile patient groups, GG/AT was 9.4 (0.9-93.6) (P= 0.05) the similar the control was statistically significant, $p<0.05$. As well as, the GA/AA revealed a statistically non-significant with the control with an odds ratio was 7.5 (0.7-78.0) (P=0.08) the GA/AT revealed a statistically non-significant with the control with an odds ratio was 1.8 (0.1-33.8) (P=0. 6) and the AA/TTrevealed a statistically non-significant with the control with an odds ratio was 5.5 (0.1-49.3) (P=0. 3) the wild type GG/AA genotype and wild type G/A allele were taken as reference.

Table 8. Genotype combination of SNPs -1213 (GGC- GAC) and --1213 (AAT - ATT) in IL10 in Patient group and Controls

IL-10 polymorphisms	Frequencies(%)		P value	Odd ratio (95% CI)
Genotype -1213 -1218	Controls (n=20)	Patient (n=20)		
Normal/ Normal GG/AA	85.0%(n=17)	45.0% (n=9)	----	1.00 (Reference)
Normal /Hetero GG/AT	5.0%(n=1)	25.0% (n=5)	0.05	9.4 (0.9-93.6)
Normal/Mutant GG/TT	0	0	----	Invalid
Hetero/ Hetero GA/AA	5.0%(n=1)	20% (n=4)	0.08	7.5 (0.7-78.0)
Hetero/ Mutant GA/AT	5.0%(n=1)	5.0% (n=1)	0.6	1.8 (0.1-33.8)
Mutant / Mutant AA/TT	0	5.0% (n=1)	0.3	5.5 (0.1-49.3)

A new mutant site has been detected in promoter gene region of interleukin-10 (-1218 ,-1213 SNP), which is not proven in international sites, and which may have an important and effective effect for most diseases in general and infertility among Iraqi women in particular who are infected with *chlamydia trachomatis* ,which is one of the causes of infection in sexually transmitted diseases and it is characteristic that they live in the cells(obligate intracellular bacteria) causing in most cases chronic damage of the fallopian tube because the patient remains infected bacteria entry in the process of hibernation which makes it difficult to be diagnosed by immunological and genetic methods sometimes. Therefore ,this mutation can be considered as a molecular indicator to assist in early diagnosis by detecting the genetic polymorphism of the IL-10 gene in women through the use of this mutation as well as other internationally proven mutations (three of which are approved and are associated with most of the immunological diseases of 592 C / A , -819 C / T and -1082 A / G), including the -1082 A / G, related to infertility. Once identified, they were published in three global database sites (NCBI, DDBG, ENB) and were encoded within the three sites (LC259134 - LC259153)

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