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Gene Expression and Polymorphism of Interleukin-4 in a Sample of Iraqi Rheumatoid Arthritis Patients

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Abstract:

It was aimed to understand the interleukin-4 (IL-4) role in etio-pathogenesis of rheumatoid arthritis (RA). Two approaches were adopted. In the first one, a quantitative expression of *IL4* gene was assessed by reverse transcription-quantitative polymerase chain reaction (RT-qPCR), and such findings were correlated with some demographic, clinical and laboratory parameters, which included gender, duration of disease, disease activity score (DAS-28), rheumatoid factors (RFs), C-reactive protein (CRP) and anti-cyclic citrullinated peptide (ACCP) antibodies. In the second approach, a single nucleotide polymorphism (SNP) of *IL4* gene (rs2243250) was inspected by DNA sequencing using specific primers. Fifty-one Iraqi RA patients (22 males and 29 females) were enrolled in the study. They were under therapy, which was a single weekly subcutaneous dose of 25 mg of etanercept (Enbrel) for a period of 3-5 years. The results of gene expression ($2^{-\Delta\Delta Ct}$) revealed an increased expression of *IL4* mRNA (Mean ± SEM: 8.247 ± 2.442), especially female patients compared to male patients (11.545 ± 3.928 *vs*. 3.537 ± 1.530; $p = 0.03$). The expression was also subjected to variations that were related to clinical and laboratory findings. With respect to *IL4* gene SNP, allele and genotype frequencies showed no significant differences between RA patients and controls. In addition, the SNP genotypes had no effect on *IL4* gene expression. In conclusion, an up-regulation of *IL4* gene expression was observed in RA patients, and it was more pronounced in female than male patients by approximately four folds, while no association between the *IL4* SNP alleles or genotypes and RA was observed.

Keywords: Rheumatoid arthritis, Interleukin-4, Gene expression, Single nucleotide polymorphism, Etanercept, Enbrel

Introduction:

Interleukin-4 (IL-4) is a pleiotropic cytokine, and its actions are generally regarded as anti-inflammatory with antagonistic effects to interferon-gamma (IFN-γ). Consequently, it down-regulates T-helper-1 (Th1) responses. IL-4 is primarily secreted by Th2 cells, but it is also produced by a subset of CD4+T cells (NKT cells), type 2 CD8+ T cells, mast cells, basophils and eosinophils (1). The receptor for IL-4 (IL-4R) is widely expressed, and therefore it influences almost all cell types that express IL-4R. In Th2 cells, IL-4 is a principle cytokine for differentiation and growth of these cells, and by this pathway, it promotes the establishment of humoral immune response (2).It has been suggested that IL-4 and its receptor may play a role in rheumatoid arthritis (RA) pathogenesis. This has been based on the evidence that depicted a reduced level of IL-4 in RA patients, and this in turn enhances Th1 mediated immune response that characterizes the autoimmune inflammatory response observed in RA patients (3). However, in a more recent study, serum level of IL-4 was reported to be significantly increased in patients with early RA compared to healthy controls. But such increased level was not correlated with some clinical and diagnostic parameters of RA (4).

In animal models of arthritis (murine type II collagen-induced arthritis), it has been demonstrated that IL-4 was associated with improvement of anti-inflammatory effects by suppressing pro-inflammatory cytokines (IL-1 and IL-6) (5). These results were previously confirmed in proteoglycan-induced arthritis in BALB/c mice, which generated

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a higher IFN-γ / IL-4 ratio (Th1 / Th2 ratio) that peaked at the onset of arthritis; however, a systemic administration of IL-4 prevented arthritis in animals and induced a switch in immune response from Th1 to Th2 (6).

Gene polymorphisms of *IL4* have also been implicated in etiology of RA. Case-control studies suggested that *IL4*gene promoter polymorphisms might be a genetic predisposing factor (5)for RA severity (7). A further study also indicated that the promoter polymorphism may be useful for assessing RA severity, and $IL4_{-590}$ TT genotype showed a significant impact on IL-4, which showed a decreased serum level (8). The SNP $IL4_{-590}$ T/C has also been associated with an increased risk to develop RA in Chinese and European patients (9). More recently, a meta-analysis of nine studies reported that the promoter polymorphism of $IL4_{-590}$ can be used as a genetic marker for evaluating RA susceptibility and severity (10).

Based on these findings, gene expression and polymorphism of IL-4 were investigated in a sample of Iraqi RA patients, who were under etanercept therapy.

Materials and Methods:

Patients:

The ethics committee at the Iraqi Ministry of Health approved the study, in which, 51 RA patients (22 males and 29 females) were enrolled, and their age range was 20 - 63 years. The diagnosis was made by the consultant medical staff at the Rheumatology Unit (Baghdad Teaching Hospital). It was based on a clinical examination, X-ray findings and laboratory tests. The diagnosis was according to the revised diagnostic criteria established by the American College of Rheumatology (ACR), 2010, which included tender and swollen joint counts, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), anti-cyclic citrullinated peptide (ACCP) antibodies, rheumatoid factors (RFs) and symptom duration (11). The RA patients were under therapy, which was a single weekly subcutaneous dose of 25 mg of etanercept (Enbrel) injection. For the purpose of comparison, 45 apparently healthy individuals (15 males and 30 females) were also enrolled in the study, and their age range was 25 - 52 years.

Gene expression of *IL4*:

The *IL4* gene expression was determined by the reverse transcription-quantitative polymerase chain reaction (RT-qPCR) technique, as previously described (12). Briefly, The GoTaq® One-Step RT-qPCR System kit (Promega, USA) was used to assess the expression. The forward and reverse primers were 5'-CCAACTGCTTCCCCCTCTG-3' and 5'-TCTGTTACGGTCAACTCGGTG-3', respectively, while those for the housekeeping gene *GAPDH* (reference gene: *glyceraldehyde-3-phosphate dehydrogenase*) were 5'-AGCCGAGCCACATCGCT-3' and 5'-CAGCCCTGGTGACCAGGC-3', respectively (13). The assessment was carried out in a 20 µl reaction volume, which consisted of 10 µl master mix, 0.5 µl RT mix, 2 µl of each primer, 5 µl RNA and 0.5 µl nuclease-free water. The following PCR cycling conditions were employed: cDNA synthesis at 37˚C for 15 minutes (1 cycle), initial denaturation at 95˚C for 5 minutes (1 cycle), followed by 40 cycles of denaturation at 95˚C (30 seconds), annealing at 60˚C (30 seconds) and extension at 72˚C (30 seconds) (Promega, USA). The double delta Ct analysis was used to assess the expression of *IL4* gene, in which *GAPDH* was the housekeeping reference gene. The results were expressed as a fold change ($2^{-\Delta\Delta Ct}$) in the expression level of target gene that was normalized to endogenous control (housekeeping gene) and relative to calibrator, which is the target gene in control subjects (14).

Gene polymorphism of *IL4*:

The Genomic DNA was extracted from a blood collected in EDTA tubes using Relia Prep TM Blood gDNA Miniprep System (Promega Corporation, USA), which was subjected to PCR amplification. The *IL4* gene SNP (rs2243250) was inspected by DNA sequencing using specific primers. The forwards (5'-ACTAGGCCTCACCTGATACG-3') and reverse (5'-GTTGTAATGCAGTCC TCCTG-3') primers were adopted from a previously published study (15). The PCR reaction was performed in a final volume of 25 µl; which included 12.5 µl Go Taq green Master mix, 0.75 µl of each primer (10 µM), 2 µl DNA sample (50 ng) and 9 µl nuclease-free distilled water. The PCR conditions were initial denaturation (95°C for 5 minutes; 1 cycle), followed by 35 cycles of denaturation (95°C for 30 seconds), annealing (60°C for 30 seconds) and extension (72°C for 30 seconds), and followed by a final extension at 72°C for 7 minutes (16). The amplified PCR fragments were subjected for agarose-gel electrophoresis (1% agarose at 5 v/cm^2 for 45 minutes) and Sanger's sequencing using ABI3730XL automated DNA sequencer (Macrogen Corporation – Korea). The genotypes were revealed by Geneious software after alignment with a reference sequence in the Gene Bank.

Statistical analysis:

Gene expression data was given as mean ± standard error (SEM). Significant differences between means were assessed by Analysis of Variance (ANOVA) followed by Least Significant Difference (LSD), in which, a probability ≤ 0.05 was considered significant. The statistical package

SPSS version 13.0 was employed to carry out these analyses. Allele frequencies of cytokine genes were estimated by the direct gene counting method, while a significant departure from Hardy-Weinberg equilibrium (HWE) was estimated using H-W calculator for two alleles (www.had2know.com/academics/hardy-weinberg-equilibrium-calculator-2-alleles.html).

Genotypes of cytokine SNPs were given as percentage frequencies and significant differences between their distributions in RA patients and controls were assessed by Fisher's exact probability (*p*), which was corrected for the number of comparisons that were made at each locus (Bonferroni Correction). In addition, odds ratio (OR) was also estimated to define the association between a genotype and RA (17). These estimations were calculated by using the WINPEPI computer programs for epidemiologists (www.brixtonhealth.com).

Results:

Gene expression of *IL4*:

The ΔCt mean of *IL4* mRNA was increased in total and male RA patients (4.600 ± 0.761 and 6.072 ± 1.184, respectively) compared to the corresponding ΔCt means in controls (2.940 ± 0.824 and 0.993 ± 0.693), but a significant difference was observed between male patients and male controls (p = 0.006). The folding expression ($2^{-\Delta\Delta Ct}$) of *IL4* mRNA was increased by 8.247 ± 2.442 folds in total RA patients, but such increase was significantly more pronounced in female patients than male patients (11.545 ± 3.928 *vs*. 3.537 ± 1.530; p = 0.03) (Table 1).

Table 1. Expression of *IL4* mRNA in rheumatoid arthritis patients and controls.

Groups		Number	ΔCt (Mean ± SEM)	LSD *p*	$2^{-\Delta\Delta Ct}$ (Mean ± SEM)	
Total	Patients	51	4.600 ± 0.761	NS	8.247 ± 2.442	
	Controls	45	2.940 ± 0.824			
Males	Patients	22	6.072 ± 1.184	**0.006**	3.537 ± 1.530	***p* = 0.03**
	Controls	15	0.993 ± 0.693			
Females	Patients	29	3.570 ± 0 .966	NS	11.545 ± 3.928	
	Controls	30	3.913 ± 1.154			

NS: Not significant ($p > 0.05$).

The increased mean of $2^{-\Delta\Delta Ct}$ showed no significant variation when RA patients were distributed to subgroups according to duration of disease; however, significant variations between means of patients distributed by DAS-28, RFs, CRP and ACCP antibodies were observed. The $2^{-\Delta\Delta Ct}$ mean was oppositely distributed as DAS-28 progressed from Low to High; it was the highest in Low DAS-28 patients (22.983 ± 3.173), and then decreased to 10.216 ± 3.906 in Medium DAS-28 patients, and a further decrease was recorded in High DAS-28 patients (3.9187 ± 1.546). Such variations were significant (p = 0.001). For RFs, sero-positive RA patients showed a decreased mean of $2^{-\Delta\Delta Ct}$ compared to sero-negative patients (2.854 ± 0.923 *vs*. 14.314 ± 4.841; p = 0.001), while an opposite observation was made in the case of CRP. Patients sero-positive for CRP showed a significant increased mean of $2^{-\Delta\Delta Ct}$ compared to sero-negative patients (11.243 ± 3.650 *vs*. 2.755 ± 0.977; p = 0.02). When RA patients distributed according to ACCP antibodies, strong sero-positive patients recorded the highest mean of $2^{-\Delta\Delta Ct}$ (15.775 ± 5.630), followed by weak and moderate sero-positive patients (4.532 ± 2.132 and 1.516 ±1.190, respectively). Such variation attended a significant level at p = 0.003 (Table 2).

Table 2. Expression fold ($2^{-\Delta\Delta Ct}$) of *IL4* mRNA in rheumatoid arthritis patients distributed by laboratory and clinical findings.

Groups		Number	$2^{-\Delta\Delta Ct}$ (Mean ± SEM)	Probability
Duration of Disease	< 5 years	14	12.545± 4.893	NS
	5 -10 years	26	7.359 ± 3.872	
	> 10 years	11	4.875 ±2.510	
Disease Activity Score (DAS-28)	Low	2	22.983 ± 3.173	**0.001**
	Medium	29	10.216 ± 3.906	
	High	20	3.9187± 1.546	
Rheumatoid Factors	Positive	27	2.854 ± 0.923	**0.001**
	Negative	24	14.314 ± 4.841	
C-reactive Protein	Positive	33	11.243 ±3.650	**0.02**
	Negative	18	2.755 ± 0.977	
ACCP antibodies	Weak positive	24	4.532 ±2.132	**0.003**
	Moderate positive	8	1.516±1.190	
	Strong positive	19	15.775 ±5.630	

NS: Not significant ($p > 0.05$)

Although the $2^{-\Delta\Delta Ct}$ means showed no significant variation between the subgroups of disease duration, gender variation was observed. Female patients at the disease duration groups < 5 and 5 -10 years showed increased means of $2^{-\Delta\Delta Ct}$ (21.186 ± 7.247 and 9.451 ± 5.840, respectively) compared to the corresponding males (1.023 ± 0.853 and 3.408 ± 1.877, respectively), and the differences were significant (p = 0.03 and 0.04, respectively). For duration > 10 years, male patients showed a non-significant increased mean of $2^{-\Delta\Delta Ct}$ compared to female patients (6.102 ± 3.864 *vs.* 2.728 ± 1.752) (Table 3).

Table 3. Expression fold ($2^{-\Delta\Delta Ct}$) of *IL4* mRNA in rheumatoid arthritis patients distributed by duration of disease and gender.

Duration of Disease		Number	$2^{-\Delta\Delta Ct}$ (Mean ± SEM)	LSD p
< 5 years	Males	6	1.023 ± 0.853	**0.03**
	Females	8	21.186 ± 7.247	
5 -10 years	Males	9	3.408 ± 1.877	**0.04**
	Females	17	9.451 ± 5.840	
> 10 years	Males	7	6.102 ± 3.864	NS
	Females	4	2.728 ± 1.752	

NS: Not significant (p> 0.05)

Gene polymorphism of *IL4*:

Agarose gel electrophoresis of the *IL4* gene PCR amplified products showed a single band of 254bp (Figure 1).

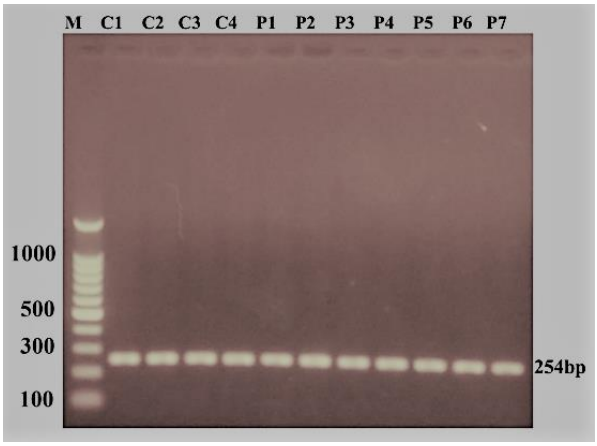

Figure 1. Gel electrophoresis of *IL4* gene PCR products (SNP rs2243250) on 1% agarose at 5 v/cm^2 for 45 minutes showing bands of 254bp molecular size (Lane M: 100bp DNA ladder; Lanes C1-C4: samples of control subjects; Lanes P1-P7: samples of rheumatoid arthritis patients).

The SNP of *IL4* gene (rs2243250 C/T) was observed to have three genotypes (CC, CT and TT) that were correspondent to two alleles (*C* and *T*) (Figure 2). These genotypes showed a good agreement with HWE in patients and controls (i.e. no significant difference between the observed and expected genotype frequencies) (Table 4). In addition, there were no significant variation between patients and controls in the distribution of these genotypes and alleles (Table 5). When the $2^{-\Delta\Delta Ct}$ mean of *IL4* mRNA was inspected in these genotypes, it was increased by 10.352 ± 4.463 folds in CT genotype compared to the homozygous CC wild genotype, but the difference was not significant (p > 0.05) (Table 6).

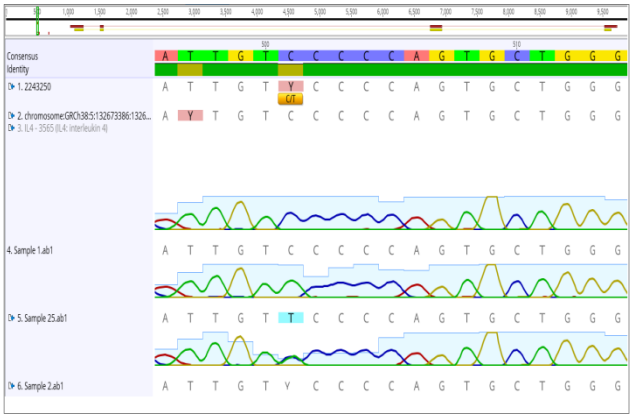

Figure 2. DNA sequence chromatogram of *IL4* gene SNP (C/T: rs2243250) showing three genotypes: CC (sample1), CT (sample 2; Y) and TT (sample 25). In addition, the reference sequence (rs2243250) is also given (Geneious software).

Table 4. Numbers and percentage frequencies (observed and expected) of *IL4* gene (rs2243250 SNP) genotypes and their Hardy-Weinberg equilibrium (HWE) in rheumatoid arthritis patients and controls.

Genotype	Rheumatoid Arthritis Patients (No. = 51)				Controls (No. = 45)			
	Observed		Expected		Observed		Expected	
	No.	%	No.	%	No.	%	No.	%
CC	37	72.5	37.1	72.8	36	80.0	36.5	81.0
CT	13	25.0	12.8	25.1	9	20.0	8.1	18.0
TT	1	2.0	1.1	2.1	ND	ND	0.5	1.0
HWE Analysis	$X^2 = 0.0132$; D.F. = 1; $p > 0.05$				$X^2 = 0.556$; D.F. = 1; $p > 0.05$			

Table 5. Statistical analysis of association between genotypes and alleles of *IL4* gene (rs2243250 SNP) and rheumatoid arthritis.

Genotype or Allele	Patients (No. = 51)		Controls (No. = 45)		Odds Ratio	95% Confidence Interval.	*p*	Bonferroni Correction
	No.	%	No.	%				
CC	37	72.5	36	80.0	0.66	0.26 - 1.70	NS	NS
CT	13	25.0	9	20.0	1.37	0.53 - 3.55	NS	NS
TT	1	2.0	ND	ND	-	-	-	-
C	87	85.3	81	90.0	0.64	0.27 - 1.55	NS	NS
T	15	14.7	9	10	1.55	0.65 - 3.72	NS	NS

p: Probability; ND: Not detected; NS: Not significant ($p > 0.05$)

Table 6. Impact of rs2243250 SNP on *IL4* mRNA expression in rheumatoid arthritis patients.

Genotype	Number	$2^{-\Delta\Delta Ct}$ (Mean ± SEM)	Probability of CC *vs.* CT
CC	37	7.729 ± 2.996	Not Significant ($p > 0.05$)
CT	13	10.352 ± 4.463	
TT	1	0.0291	

Discussion:

An up-regulation of *IL4* gene expression was observed in RA patients, and it was more pronounced in female patients than male patients by approximately four folds ($2^{-\Delta\Delta Ct}$ = 11.545 and 3.537, respectively). In agreement with such findings, it has been reported that serum level of IL-4 was significantly increased in South Indian RA patients. A higher expression of *IL4* gene was also reported in the patients than in healthy controls, but the difference reached no significant level (13). The results of both studies may contradict the general functional role of IL-4 in pathogenesis of autoimmune diseases, including RA; although it has been suggested that IL-4 is not be involved in the deleterious effects of the disease (18). However, it is believed that IL-4 can exert anti-inflammatory effects in autoimmune diseases, which are due to abnormal immunological functions. These diseases are dependent on activation of Th1 cells and monocytes, which produce pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-12, and TNF-α) to mediate their effects, as demonstrated in RA patients (19). In this context, IL-4 is able to inhibit the formation of Th1 cells by directing the differentiation of Th0 cells into Th2 cells (20). In addition, it is well-known that IL-4 is able to inhibit pro-inflammatory cytokines production by monocytes, and at the same time induces the production of anti-inflammatory factors (IL-1R antagonist and soluble TNF-α receptors) (21). It is also interesting to notice that IL-4 production has either not been detected or low levels were produced by cultured cells from the synovium of RA patients, and moreover, very low levels of IL-4 have been found in the synovial fluid of patients (22). Animal model studies confirmed the importance of IL-4 in pathogenesis of RA, and a more aggressive disease was observed in IL-4 deficient mice than in wild type mice. Moreover, gene therapy using dendritic cells genetically-engineered to express IL-4 was demonstrated to reduce inflammation and ameliorate the destruction of joints in mouse models of RA. Based on these findings, it has been suggested to use IL-4 as a good complement treatment in RA patients receiving anti-TNF-α therapies (5). However, such suggestion may also justify the increased expression of *IL4* gene in present RA patients, because they were under etanercept therapy, which is an anti-TNF-α agent, and blocking TNF-α may render IL-4 to have an increased expression that was decreased due to RA or other factors that participate in the disease pathogenesis (23).

The expression of *IL4* gene ($2^{-\Delta\Delta Ct}$) also showed significant variations in RA patients distributed by DAS-28, RFs and CRP, and ACCP antibodies. With respect to DAS-28, the $2^{-\Delta\Delta Ct}$ showed a gradual decreased mean as patients progressed from Low to High DAS-28; this means

that the expression of *IL4* was correlated with severity of disease. There is no direct evidence that can support such finding, but it has been reported that serum level of IL-4 showed a significant increased level in RA patients at the earlier stages of disease (less than one year of disease duration) (24); however, the authors reported no correlation between IL-4 and DAS-28, and a similar conclusion was reached in a further group of RA patients (13). In a more recent investigation, a positive correlation between IL-4 plasma level and DAS-28 in RA patients was reported (25). However, no correlation between serum IL-4 level and systemic onset juvenile idiopathic arthritis has also been reported (26). Accordingly, the correlation between IL-4 and disease activity is still a matter of controversy, and this is probably related to the homogeneity of investigated patients and the therapy that are receiving.

For RFs, sero-negative RA patients showed an increased expression of *IL4* gene compared to sero-positive patients. Inspecting other inflammatory markers in relation to *IL4* gene expression revealed that patients sero-positive for CRP and strong sero-positive patients for ACCP antibodies recorded a significant increased expression compared to sero-negative patients. These findings may suggest that the mechanism of RA pathogenesis is different in RA patients, and it might be subjected to the status of inflammatory markers (RF, CRP and ACCP antibodies). Mariaselvam *et al*. (13) adopted a similar approach to find a correlation between *IL4* gne expression and RF, CRP and ACCP antibodies, and their results may share the theme of present study and revealed a positive correlation with high sensitive CRP (hsCRP) and duration of disease in South Indian patients. With respect to duration of disease, the present results also reveal that female patients at the disease duration subgroups < 5 and 5 -10 years show a significant increased expression of *IL4* gene compared to the corresponding males. However, in an earlier further investigation, no clinically important differences were found between RA patients positive and negative for ACCP antibodies, and both groups of patients showed a similar pattern for synovial fluid level of IL-4, disease duration, ESR and CRP (27).

With respect to *IL4* gene polymorphism (rs2243250 C/T), allele and genotype frequencies showed no significant variations between RA patients and controls. Many previous investigations have examined the association between *IL4* gene polymorphisms and RA, but their findings are conflicting. The $IL4_{-590}$ SNP was examined in RA patients and overall analysis of genotypes revealed no significant difference between patients and control subjects. However, it was found that patients with the active form of disease were more frequently presented with the genotypes CT and TT. Accordingly, the authors suggested that the *T* allele may be a genetic risk factor for severity of RA (7). A further study also indicated that the $IL4_{-590}$ *T* allele may be useful marker for assessing RA severity, and in addition TT genotype was associated with a decreased serum level of IL-4 (8). In Chinese RA patients, the TT and CT genotype frequencies were reported to be significantly increased compared to controls, while CC genotype frequency was significantly decreased. Accordingly, *T* allele was suggested to be a risk factor for RA in Chinese patients (9). Logistic regression analyses also showed that carriers of *IL4 T* allele (rs2243250) had a significantly increased risk for RA (28). More recently, a meta-analysis of nine studies under three genetic models (co-dominant, dominant, recessive and allele models) reported that overall analysis revealed strong associations that correspond to each genetic model; *C vs*. *T* in allele model, CC *vs*. CT + TT in dominant model and CC + CT *vs*. TT in recessive model (10).

To assess the impact of *IL4* SNP (rs2243250) on the expression of *IL4* gene in RA patients, the $2^{-\Delta\Delta Ct}$ mean was inspected in patients. It was observed that CT genotype showed an increased expression compared to CC genotype, but the difference was not significant. Previous studies also suggested that $IL4_{-590}$ SNP genotypes may affect the serum level of IL-4; although the observations were not consistent (29).

In conclusion, an up-regulation of *IL4* gene expression was observed in RA patients, and it was more pronounced in female than male patients by approximately four folds, while no association between the *IL4* SNP alleles or genotypes and RA was observed.

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Conflicts of Interest: None.

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التعبير الجيني وتعدد الاشكال الوراثي للبين ابيضاض-4 في عينة من مرضى التهاب المفاصل الرثوي العراقيين

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الخلاصة:
كان الهدف بيان دور البين ابيضاض-4 في امراضية التهاب المفاصل الرثوي. اعتمدت طريقتين؛ وتم في الأولى تحديد التعبير الكمي لجين البين ابيضاض-4 بطريقة الاستنساخ المعاكس الكمي-تفاعل انزيم البلمرة المتسلسل، ودرست العلاقة بين هذه النتائج وبعض المعايير الديموغرافية والسريرية والمخبرية والتي تضمنت الجنس ومدة المرض وعامل نشاط المرض والعامل الرثوي والبروتين التفاعلي-سي ومضاد ببتيد الستروليناید الدوري. اما في الطريقة الثانية، فقد تم التحري عن تعدد اشكال النيوكليوتيدة المفرد (SNP) لجين البين ابيضاض-4 (rs2243250) وذلك باستخدام تقنية تسلسل الحامض النووي وباستخدام بوادئ متخصصة. شملت الدراسة 51 مريض من مرضى التهاب المفاصل الرثوي العراقيين (22 ذكور و29 اناث)، والذين كانوا تحت العلاج بواقع حقنة أسبوعية تحت الجلد (25 ملغم) من الدواء Enbrel لفترة 3-5 سنوات. اظهرت نتائج التعبير الجيني ($2^{-\Delta\Delta Ct}$) زيادة ملحوظة في تعبيرة (المعدل ± الخطأ القياسي: 8.247 ± 2.442) خصوصا في المرضى الاناث مقارنة بالذكور (11.545 ± 3.928 مقابل 3.537 ± 1.530؛ الاحتمالية = 0.03). وخضع هذا التعبير لتغايرات لها علاقة بالمعايير السريرية والمخبرية. اما بخصوص تعدد اشكال النيوكليوتيدة المفردة لجين البين ابيضاض-4، فلم تظهر تكرارات الانماط الاليلية والجينية اختلافات ذات دلالة إحصائية بين مرض داء المفاصل الرثوي والسيطرة. وفضلا عن ذلك، فلم يكن هناك تأثير للانماط الجينية على التعبير الجيني لجين البين ابيضاض-4. وفي الاستنتاج فقد لوحظ زيادة في التنظيم لتعبير جين البين ابيضاض-4 في مرض التهاب المفاصل الرثوي، وكان ذلك اكثر وضوحا في المرضى الاناث مما هو عليه في المرضى الذكور بحوالي اربع مرات، في حين لم تلاحظ مصاحبة بين الانماط الاليلية والجينية لتعدد اشكال النيوكليوتيدة المفردة لجين البين ابيضاض-4 والتهاب المفاصل الرثوي.

الكلمات المفتاحية:التهاب المفاصل الرثوي، بين ابيضاض-4، تعبيير جيني، عدد اشكال النيوكليوتيدة المفردة، Etanercept، Enbrel