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Il-4Rα, TNF-α,TNF-α Receptor, and CD4 Enhancer Genes Polymorphisms in Rheumatoid Arthritis Saudi Female Patients

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Abstract:*We aimed to investigate the role of IL-4Rα, TNF-α, its receptor, and CD4 enhancer gene polymorphisms in susceptibility and severity of rheumatoid arthritis (RA).One hundred and fifty RA patients and 100 controls were enrolled in the study. Genotyping of IL-4Rα I50V (rs1805010) and IL-4Rα Q576R (rs1801275), TNF-α -308 G/A polymorphism in the promoter region of the TNF-α gene and −196M/R polymorphism of TNFRII gene, CD4-11743 and CD4-10845 gene polymorphismwas determined by PCR-RFLP.IL-4Rα 50IV genotype was significantly more frequent in patients with RA than in controls (OR: 1.97, 95% CI: 1–3.7, P: 0.035). Subjects with IL-4Rα 50V/V genotype were significantly more likely to have erosive arthropathy (OR: 2.6, 95% CI: 1.1–6.1, P: 0.02). The frequencies of IL-4Rα Q576R genotype were significantly decreased in patients with erosive RA compared to patients with non erosive RA (31.6%versus 48.2%, OR: 2.7, 95% CI: 1–7.7, P: 0.04).Our findings suggested that the A allele of TNF-α and TNFRII 196M/R polymorphism were associated with RA susceptibility. Subjects with the CC genotype of CD4-11743 were significantly more likely to develop RA (OR = 2.7, P = 0.03) and more likely to have severe RA (OR = 2.7, P = 0.024). Carrier of A allele of CD4-10845 was significantly more likely to develop severe RA (OR = 3.7, P =0.000).In conclusion,IL-4Rα,TNF-α and its receptor, CD4 enhancer gene polymorphisms were associated with susceptibility to RA and may be helpful in early detection of erosive RA.*

Keywords:*Rheumatoid arthritis (RA), IL-4Rα,TNF-α, TNFRII, CD4, Gene polymorphism.*

I. Introduction

Rheumatoid arthritis (RA), one of the most common systemic autoimmune diseases characterized by chronic joint inflammation and subsequent joint destruction [1]. It has a worldwide prevalence of approximately 1% and is always observed to affect women 2–3 times more frequently than men [2]. Both environmental and genetic factors contribute to this pathogenesis [3]. Previous studies have shown that T cells play an important role in the development of such autoimmune disease [4]. The balance between regulatory T cells and proinflammatory effectors' T cells has been shown to be of critical importance in the development and persistence of autoimmune diseases [5].

CD4+ T cells that stimulate monocytes, macrophages, synovial fibroblasts, and other cells to produce cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6, IL-15, IL-17, and metalloproteinase that produce tissue damage [6] play the main role in the development of most human autoimmune diseases as RA [7]. This cytokines play an important role in RA pathogenesis. There is substantial evidence to support the involvement of lymphocytes in the pathogenesis of RA, including the presence of CD4+ T cells in RA synovium. The CD4 receptor is glycoprotein expressed on the surface of helper T cells colocalizes with the T-cell receptor (TCR) and major histocompatibility complex (MHC) class II molecules and interacts with the non-antigen-binding regions on the MHC class II molecule in antigen recognition [7,8].Theoretically, any malfunction of the CD4 receptor could potentially allow for improper activation and proliferation of CD4+ T cells [9]. Inhibition of CD4–MHC class II interaction severely impairs the response of T cells to antigen exposure, and low CD4 cell surface expression on the lymphocytes results in impaired TCR response to low-level antigenic stimulation [10]. The human CD4 gene maps to chromosome 12p13 in a gene-dense region [11] and its expression is regulated primarily at the transcriptional level [12]. The CD4 enhancer and promoter seem to be the major regulatory regions for CD4 transcription in T cells [13]. Therefore, an analysis of the CD4 enhancer polymorphism can provide insights into regulation of its expression. Clearly, the molecular

mechanisms controlling CD4 gene expression are very complex and are controlled by many different signals as the thymocyte develop [13].

Anti-inflammatory mechanisms play important role to control the auto reactivity. These mechanisms include the production of IL-10, IL-13, IL-4, and transforming growth factor-ß, as well as antagonists of the mediators of inflammation such as the IL-1 receptor antagonist (IL-1Rα) and soluble receptors of TNF-α [14]. IL-4 is produced by Th2 cells, basophils, eosinophils, and mastocytes and promotes the change of immunoglobulin production of B cells from IgM to IgE and IgG4 [15]. It has stimulatory and inhibitory effects, inducing differentiation of Th0 to Th2 cells, and among its anti-inflammatory processes are down regulation of pro-inflammatory cytokines such as TNF-α, IL-1ß, IL-6, and interferon-γ (IFN-γ), inhibition of the proliferation of fibroblastic cells of the articular synovium, and diminution of bone resorption[16]. It has been established that synovial concentrations of IL-4 are low or absent in patients with RA [17]. High levels of intra-articular IL-4 induce clinical improvement of the arthritis [18]. Moreover, administration of monoclonal antibodies against IL-4 in a mouse model of collagen-induced arthritis resulted in greater severity of disease [19,20].

IL-4 exerts its biological activity by binding to the target cell receptor IL-4R [21]. IL-4R is composed of two subunits: an α subunit that bind IL-4 and transduces its growth promoting and transcription activating functions [22] and a γ subunit that is common to several cytokine receptors and amplifies signaling of the α subunit [23]. The gene for IL-4 has been mapped to the q arm (q23-31) of chromosome 5 [22]. A functional polymorphism representing C-to-T substitution at position -590 has been recently described in the promoter region of IL-4 [19]. Another polymorphism has been located in the third intron, and is composed of a variable number of tandem repeats (VNTR) of a 70-bp [23]. IL4Rα is located at the short arm of chromosome 16p12.1. Several polymorphisms have been described in the codifying and in the non-codifying regions of the gene [24]. It has been established that polymorphisms located in the +148 codifying region (transition A/G, Ile50Val) of the extracellular domain [25] and in the +1902 codifying region (transition A/G, Gln576Arg (Q576R))of the intracellular domain affect the binding of its ligand or the intracellular signaling, respectively [26].

TNF-α is a multifunctional cytokine with potent pro-inflammatory effects through stimulation of T cell, up regulation of proteolytic enzymes, and increase secretion of prostaglandin and chemokines. TNF-α is a major factor involved in the RA inflammatory state [27]. The TNF-α gene is a member of TNF super family located within the class II region of the human MHC on chromosome 6p21 [28]. Promoter polymorphisms within TNF-α genes have been reported in inflammatory and infectious diseases [29]. Promoter polymorphisms at TNF-α have been associated with disease susceptibility, or severity of joint damage and autoantibody production in RA in different populations. Several TNF-α polymorphisms have been studied such as genotype 308A/G polymorphisms because of their association with increased TNF-α secretion and their implication in susceptibility of several auto-immune disease such RA [30]. The pleiotropic biological activities of TNF-α are mediated by its binding to TNF receptors (TNFR) Type I and II [31]. Both are expressed in synovial fluid and cartilage in RApatients[32]. Several studies, upon the gene polymorphism at 169 M/R in TNFR type II showed that, this polymorphism has been implicated in severity in RA [33].This work was done to evaluate the association of IL-4Rα, TNF-α, and CD4 enhancer genes polymorphism in development and severity of RA in female Saudi patients.

II. Subjects And Methods

2.1. Subjects

The study included 150 Saudi female RA patients. These patients diagnosed according to the criteria of American College of Rheumatology. Subjects included in the study underwent routine biochemical blood analysis and x-rays of the hands and feet. The evaluation of subjects included physical examination, with particular focus on the pattern of joint involvement, the presence of nodules and other extra-articular features (such as vasculitis, anemia, subcutaneous nodules, vasculitis manifestation, and organ involvement), and laboratory features such as rheumatoid factor (RF). Disease activity has been determined on the basis of defined parameters (the number of swollen and tender joints, ESR, Health Assessment Questionnaire (HAQ), and C-reactive protein (CRP) and a global physician's assessment. Disease severity hasbeen determined on the basis of defined parameters (RF and X-ray erosion). Patients deemed to have an erosive arthropathy if one or more definitive erosions appear in any of the peripheral joints that have been identified to be predictive of disease progression.

One hundred healthy Saudi women used as a healthy control group. They never had any signs or symptoms of RA, other arthritis, or joint diseases (pain, swelling, tenderness, or restriction of movement) at any site based on their medical history and examination.

2.2. Collection of Blood Samples

Eight mLof venous blood was drawn from each individual of the two groups under complete aspect condition after an overnight fasting. Three mL of blood was collected in EDTA containing tube for separation of peripheral blood mononuclear cells (PBMCs) for determination of TNF-α and its receptor, IL-4Rα, and CD4 enhancer genotypes. The other 3 ml of blood were collected in anticoagulant-free tubes used for separation of serum to detect RA and CRP, TNF-α, IL-4R, and CD4 levels. Two mL was collected in ESR tube.

2.3. Methods

2.3.1Biochemical analysis

Blood samples were drawn from all subjects after an overnight fast. Sera were separated immediately and stored at -20°C. CRP, IL-4Rα, TNF-α and CD4 are assayed by high-sensitivity enzyme-linked immunosorbent assay (ELISA). IgM RF was measured by latex agglutination. RF was considered positive above 20 IU/ml.

2.3.2 Isolation of DNA

Genomic DNA was extracted from EDTA whole-blood sample using a spin column method according to the protocol (QIAamp Blood Kit; Qiagen GmbH, Hilden, Germany).

2.3.3 Amplification of two IL-4Rα، polymorphic sites

The subjects were genotyped for IL-4Rα I50V and IL-4Rα Q576R polymorphisms by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Twenty-five micro liters of reaction mixture contains genomic DNA samples (100 ng), 200 µmol/L dNTPs,1.5 mM $MgCl_2$, 1X Taq polymerase buffer, 50 pmol ofeach primer, and 0.5 unit of Taq DNA polymerase(Amersham, Paris, France) [34,35]. For IL-4Rα I50V polymorphism the region surrounding the polymorphism was amplified with following forward primers 5'-GGCAGGTGTGAGGAGCATCC-3' and reverse primer 5'-GCCTCCGTTGTTCTCAGGGA-3'. PCR was performed at 93 °C for 5 min, followed by 36 cycles at 93 °C for 60 sec, at 60 °C for 60 sec, and 72 °C for 60 sec. Digestion of 273 bp-amplified products with RsaI yielded 273 bp for I allele and 254-bp fragment when V allele was present. While for IL-4Rα Q576R polymorphism, the region surrounding the polymorphism was amplified with following forward primers 5'-GCCCCCACCAGTGGCTACC-3' and reverse primer 5'-GCCTTGTAACCAGCCTCTCCT-3'. PCR was performed at 94 °C for 5 min, followed by 40 cycles at 94 °C for 30 sec, at 55 °C for 30 sec, and 72 °C for 60 sec. Digestion of 123 bp-amplified products with MspI yielded 107- and 16-bp fragments for Q allele and 89-, 18- and 16-bp fragments from the R allele. Quality control measures include blinded analyses, replicates of 10% of samples, and positive controls (blood-derived DNA from all known genotypes), and negative controls for contamination (no DNA) were run routinely with patient samples.

2.3.4 Amplification of -308G/A promoter polymorphism of the TNF-α gene

The subjects were genotyped for -308G/A promoter polymorphism of the TNF-α gene by PCR–RFLP as described previously [36]. the region surrounding the polymorphism was amplified with following forward primers 5'-TTCCTGCATCCTGTCTGGAA-3' and reverse primer 5'-TTCCTGCATCCTG-TCTGGAA-3'. PCR was performed at 93°C for 5 min, followed by 36 cycles at 93 °C for 60 s, at 60 °C for 60 s, and 72 °C for 60 s. A final extension step was carried out at 72°C for 5 min. After RFLP analysis using enzyme BsmFI in 65 °C for 3 h, striking on 2% agarose (Serva) with ethydium bromide and translucence by ultraviolet light, three genotypes were detected: GG (137 + 103 + 88 bp), GA (191 + 137 + 103 + 88 bp), and AA (191 + 137 bp).

2.3.5 Amplification of TNFRII polymorphism

The subjects were genotyped for TNFRII exon 6 polymorphism by PCR–RFLP as described previously [37]. The region surrounding the polymorphism was amplified with following forward primers 5'-ACTCTCCTATCCTGCCTGCT-3' and reverse primer 5'-TTCTGGAGTTGGCTGCGTGT-3'. PCR was performed at 95 °C for 5 min, followed by 35 cycles at 95 °C for 60 s, at 57 °C for 60 s, and 72 °C for 60 s. A final extension step was carried out at 72 °C for 5 min. After RFLP analysis using enzyme NlaIII in 65 °C for 3 h, striking on 2% agarose with ethydium bromide and translucence by ultraviolet light. The 242-bp PCR product was uncleaved in the 196R allele and cleaved into two fragments of 133- and 109-bp in the 196M allele.

2.3.6 Detection of two CD4 polymorphic sites

CD4-11743 A/C and CD4-10845 A/G polymorphisms were analyzed by PCR–RFLP method as previously describe by Sui-Foon et al [38]. The region surrounding CD4-11743 A/C polymorphismwas amplified with following forward primers 5'-TCAGATATTCTCTGCTCAGCCCA-3' and reverse primers 5'-TTC CAGTCTGAAAAAAGTGG-3'.The amplification protocol comprised initial denaturation at 95°C for 5

min, 18 cycle at 95°C for 30 s, touchdown 60-51°C for 30 s, and 72°C for 30 s, 17 cycles at 95°C for 30 s, 51°Cfor 30 s, and 72°C for 30 s, and final extension at 72°C for 7 min. The 302-bp PCR products were digested with MwoI for 3 h at 37°C yielded 302-bp for A allele and 205- and 97-bp fragments when C allele was present. The region surrounding the CD4-10845 A/G polymorphism was amplified with following forward primers 5'-GAAATGAGAAGTAGCACACAGT-3' and reverse primers 5'-AAAAGTTAAGCAGAATCAGGC-3'.Two fragments measuring 202- and 98-bp would be present if the product is able to be excised. The uncut band showed up as a 300-bp length on the gel. The 300-bp PCR products were digested with HaeII for 3 h at 37°C yielded 300-bp for A allele and 202-bp and 98-bp fragments when G allele was present. Quality control measures include blinded analyses, replicates of 10% of samples, and positive controls (blood-derived DNA from all known genotypes), and negative controls for contamination (no DNA) were run routinely with patient samples.

2.3.7 Statistical analysis

The Statistical Package for Social Science (SPSS) program (release 7.5.1; SPSS Inc, Chicago, IL, 1996) will be used for data analysis.

III. Results

Demographic, clinical, and laboratory characteristics of the RA patients are shown in Table 1.

3.1 IL-4Rαpolymorphisms genotypes and alleles distribution in RA and control

The genotype frequencies of the IL-4Rα I50V and IL-4RαQ576R were in agreement with Hardy–Weinberg equilibrium in all groups. In RA patients, the frequencies of IV genotype were significantly increased compared to control group (58.7% versus 41.4%). Subjects with IV genotype were significantly more likely to have RA (OR = 1.97, 95% CI = 1–3.7, P = 0.035). Whereas, the allele frequencies of IL-4Rα were not significantly different between control and patients group (Table2).

3.2 Association of IL-4Rα with bone erosion in RA

In patients with erosive RA the frequencies of VV genotype were significantly increased compared to patients with non erosive RA (26.7% versus 17.9%). Subjects with VV genotype were significantly more likely to have erosive arthropathy (OR = 2.6, 95 % CI = 1.1–6.1, P = 0.02). The frequencies of QR genotype were significantly decreased in patients with erosive RA compared to patients with non erosive RA (31.6% versus 48.1, OR= 2.7, 95 % CI = 1–7.7,P = 0.04) (Table 3).

3.3 Association of IL-4R α with RF in RA

Carriers of V allele of IL-4RαI50V and Q allele of IL-4RαQ576R were significantly more likely to be RF-positive (> 20 IU/ml)(OR = 2.6, 95 % CI = 1.5–4.7, P = 0.001, CI = 3.2–11.5,P = 0.000), respectively and consequently develop severe RA (Table 4).

3.4 Disease activity parameters in relation to IL-4Rα genotypes

Patients with VV genotype show significant increase in the levels of RF as compared to the other two genotypes. The disease activity in patients carrying VV genotype was increased more than two-fold as compared to IV and more than three-fold as compared to II genotype. Also, QQ genotype shows significant increase in the levels of RF as compared to the other two genotypes. The disease activity in patients carrying QQ genotype was increased more than three-fold as compared to QR and more than 17-fold as compared to RR genotype. On the contrary, none of the other disease activity parameters show any correlation with VV or QQ genotypes (Table 5).

3.5 TNF-α -308 and TNFRII -196 MR genotypes frequencies

The frequencies of the GG, GA, and AA genotypes of -308G/A polymorphism of the TNF-α gene (rs 1800629), were 90%, 7%, and 3% in controls, 75%, 21.23%, and 4% in RA patients. There was a significant difference in the genotypes frequencies polymorphism of the TNF-α promoter -308G/A between control and RA group ($p < 0.05$) (Table 6). GA genotype was associated with a significantly increased risk of RA group as compared to control group (OR=3.78, 95% CI(0.86-16.6)).

The frequencies of the MM, MR, RR genotypes of TNFRII gene polymorphism were 48%, 43%, and 9% in control, and 33%, 53%, and 14% in RA patients. There was a significant difference in the genotypes frequencies polymorphism of the TNFRII 196MR polymorphism between control and RA group ($p < 0.05$) (Table 6). RR genotype was associated with a significantly increased risk of RA group as compared to control group.

3.6TNF-α -308 and TNFRII -196MR allele frequencies

The frequencies of the G and A alleles of -308G/A TNF-α gene were 94 % and 6% in control, 87% and 13% in RA patients. There was a significant difference in the allele frequencies polymorphism of the TNF-α promoter -308G/A polymorphism between control and RA group ($p < 0.05$) (Table 7). The A allele was associated with a significantly increased risk of RA (OR=1.00, 95% CI (0.03-29.08)). The frequencies of the M and R allele of TNFRII gene polymorphism were 70%, and 30% in control, and 58 %,and 42% RA patients,the R allele was associated with a significantly increased risk of RA (OR=3.19, 95% CI (1.11-9.14).There wasa significant difference in the allele frequencies polymorphism of the TNFRII196MR polymorphism between control and RA group ($p < 0.01$) (Table 7).

3.7 Serum TNF level

There was a significant difference in TNF-α serum level among control and RA groups ($p < 0.001$) (Table 8).

3.8 The relation between TNF-α -308 and TNFRII -196 MR genotypes and TNF-α level

For TNF-α-308, there is a significant difference in TNF-α values among the all genotype GG, GA, and AA in RA patient($p<0.01$)(Table 9).For TNFRII -196MR, there is no significant difference in TNF-α values among the all genotype MM, MR, and RR in RA patient(Table 9).

The genotype and allele frequencies of the CD4-11743A/C and CD4-10845A/G polymorphisms of patients with RA and controls are shown in (Table 10).The frequencies of CC genotype of CD4-11743A/C were significantly increased in patients with RA compared with control group. Subjects with the CC genotype were significantly more likely to develop RA (OR = 2.7, 95% CI = 1–6.8, P = 0.03).However, there were no significant differences betweenCD4-10845 polymorphisms in RA and control group.

Association of CD4 genotypes and allelic frequencies with RF in RA patients is shown in (Table 11).The frequencies of CC genotype of CD4-11743A/C were significantly increased in patients with RFnegative compared with RFpositive group (58% vs. 41%, P = 0.024). Subjects with the CC genotype were significantly more likely to have sever RA (OR = 2.7, CI = 1.1–6.7, P = 0.024). The frequencies of GG genotypes of CD4-10845 polymorphisms were significantly increased in patients with RF negative compared with RF positive patients (64 vs. 6%, P = 0.001). Carrier of A allele of CD4-10845 was significantly more likely to develop sever RA (OR = 3.7, P = 0.001).

Parameters of disease activity in relation to CD4 genotypes are shown in (Table 12).There was a significant increase in number of tender joint in patients carry CC genotype as compared with patients carry AA genotype of CD4-11743 and also significant increase in number of tender joint in patients carry GG genotype as compared with patients carry AA genotype ofCD4-10845. There was a significant decrease in RF inpatients carry AG and GG variants when compared with those carry AA genotype of CD4-10845A/G polymorphism.

IV. Tables

Table 1: Patient's characteristics

RA patients	
No.	150
Age	44.7± 8.1
Disease duration	9.6± 6.8
No. swollen joint	6.1 ± 3.9
No. tender joints	14.0± 6.2
ESR (mm/h)	46.0± 31.8
RF	78.2± 81.1
CRP (lg/ml)	17.2± 15.3

Table 2: Distribution of IL-4R-α genotypes in RA patients and control group

Genotypes		Controls (N = 100), N (%)		RA (N = 150), N (%)		OR	Confidence interval	P
IL-4Rα I50V	II	44	(39.6)	40	(26.7)			
	IV	46	(41.4)	88	(58.7)	1.97	(1–3.7)	0.035
	VV	21	(18.9)	32	(21.3)	1.9	(0.9–4.4)	> 0.05
	V allele	89	(44.5)	140	(46.7)	0.7	(0.4–1.2)	> 0.05
IL-4Rα Q576R	RR	17	(17)	16	(10.7)			
	QR	53	(53)	48	(32)	1.4	(0.6–3.5)	> 0.05
	QQ	44	(44)	56	(37.3)	0.4	(0.2–1.2)	> 0.05
	Q allele	141	(70.5)	160	(53.8)	0.7	(0.4–1.3)	> 0.05

Table3: Association of IL-4R-**α** with bone erosion in rheumatoid arthritis

Genotypes		Erosive (N = 50), N (%)		Non-erosive (N = 100), N (%)		OR	Confidence interval	P
IL-4Rα I50V	II	8	(16.7)	33	(30.4)			
	IV	26	(56.7)	55	(51.8)	1.9	(0.9–3.9)	> 0.05
	VV	13	(26.7)	18	(17.9)	2.6	(1.1–6.1)	> 0.02
	V allele	55	(44.5)	94	(43.8)	0.6	(0.4–1.1)	> 0.05
IL-4Rα Q576R	RR	7	(55)	7	(7.1)			
	QR	16	(31.6)	51	(48.2)	2.7	(1–7.7)	> 0.04
	QQ	27	(55)	48	(44.6)	0.6	(0.2–1.8)	> 0.05
	Q allele	70	(70.8)	149	(68.8)	1.1	(0.6–2)	> 0.05

Table 4: Association of IL-4R-**α** with RF in rheumatoid arthritis

Genotypes		RF-negative (N = 50), N (%)	RF-positive (N = 100), N (%)	OR	Confidence interval	P
IL-4Rα I50V	I allele	70 (68.5)	85 (44.9)	2.6	(1.5–4.7)	0.001
	V allele	30 (31.5)	115 (55.1)			
IL-4Rα Q576R	R allele	60 (57.4)	35 (17.8)	6	(3.2–11.5)	0.000
	Q allele	40 (42.6)	165 2.2)			

Table 5: Disease activity parameters in relation to IL-4R-α genotypes.

	IL-4R-**α** I50V			IL-4R-**α** Q576R		
	II (38)	IV (80)	VV (32)	QQ (72)	QR (64)	RR (14)
ESR (mm/h)	48.1± 33	47.1 ± 36.6	41.6 ± 21.5	46.4 ± 20.7	47.2± 41.4	54.5 ± 42.7
RF (IU/mL)	45.7± 75[a]	59.1 ± 67[a]	157.6 ± 83	122.6 ± 85	40.6± 42.3§	7 ± 2.1§
CRP (mg/l)	18.2± 17.6	13.2 ± 14.9	1.8 ± 9.8	17.8 ± 16.8	13.9± 10.6	17 ± 19
No. of swollen joints	4.9± 4.8	5.1 ± 4.4	3.8 ± 3.3	4.9 ± 3.8	5.7 ± 5.1	3 ± 2.1
No. of tender joint	13.2± 6	12.2 ± 7.5	11.5 ± 7.2	12.93 ± 7.2	13.4± 6.6	13.6 ± 8.7
HAQ	1.5 ± 0.8	1.4 ± 0.7	1.3 ± 0.8	1.6 ± 0.9	1.4 ± 0.6	1.1 ± 0.7

CRP: C-reactive protein; HAQ: Health Assessment Questionnaire; IL-4R-**α**: IL4-receptor-**α**; RA: rheumatoid arthritis; RF: rheumatoid factor.[a] Significant difference from VV, [§] Significant difference from QQ

Table 6: The genotype frequencies of -308G/A of the TNF-α gene and TNFRII 196MR among the controls and the RA patients

Polymorphism	**Cases n = 150**	**Controls n = 100**	**OR (95% Cl)**	**X^2**	**P**
TNF-α -308				6.08	<0.05
GG	113(75)	90 (90)	0.24 (0.07- 0.83)		
GA	31 (21)	7 (7)	3.78 (0.86-16.6)		
AA	6 (4)	3 (3)	1.52 (0.17-12.99)		
TNFRII 196MR				6.46	<0.05
MM	49 (33)	48 (48)	0.46 (0.23-0.92)		
MR	80(53)	43 (43)	1.5 (0.74-3.01)		
RR	21 (14)	9 (9)	2.34 (0.69-8.34)		

Table 7: The allele frequencies of -308G/A of the TNF-α gene and TNFRII 196MRamong the controls and the RA patients

Polymorphism	RA 150	Controls 100	OR (95% Cl)	X^2	P
TNF-α -308				5.14	<0.05
G	260 (87)	188 (94)	0.47 (0.19-1.14)		
A	40 (13)	12 (6)	1.00 (0.03-29.08)		
TNFRII 196MR				6.02	<0.01
M	174 (58)	140(70)	0.55 (0.32-0.93)		
R	126 (42)	60 (30)	3.19 1.11-9.14)		

Table 8: Serum TNF-α levels in the studied groups

Group	**n**	**Mean±SD serum TNF**	**T**	**P**
Control	100	26.99±5.6	6.66	<0.001
Rheumatoid Arthritis	150	36.92±7.91		

Table 9: Serum TNF-α levels in the different genotypes in all the studied groups

Group	N 150	Mean±SD serum TNF	F	P
TNF-α 308				
GG	114	31.70±6.74	4.818	<0.01
GA	28	24.01±6.2		
AA	8	39.18±7.88		
TNFRII 196MR				
MM	48	39.99±8.21	2.09	0.119
MR	78	38.89±7.82		
RR	24	35.01±6.12		

Table 10: Distribution of CD4 genotypes in RA patients and control group

	Controls (No. = 100)		RA (No. = 150)		OR	Confidence interval (CV)	P
	No.	%	No.	%			
CD4-11743							
AA	16	16	10	6			
AC	40	40	45	30	0.6	0.2–1.6	0.3
CC	46	46	95	64	2.7	1–6.8	0.03
C allele	124	62	221	74	0.6	0.3–1	0.06
CD4-10845							
GG	13	13	15	10			
AG	38	38	56	37	0.9	0.4–2.3	0.9
AA	49	49	79	53	0.8	0.4–2.1	0.7
A allele	148	32	200	67	0.9	0.5–1.6	0.7

Table 11: Association of CD4 genotypes with RF in rheumatoid arthritis

	RF negative (No. = 50)		RF positive (No. = 100)		OR	Confidence interval (CV)	P
	No.	%	No.	%			
CD4-11743							
AA	4	8	14	14			
AC	15	30	42	42	0.7	0.28–1.8	<0.4
CC	31	62	44	44	2.7	1.1–6.7	<0.024
C allele	72	72	128	64	1.8	1–3.4	<0.04
CD4-10845							
GG	32	64	6	6			
AG	14	28	28	28	8.7	3.4–22.3	<0.001
AA	4	8	66	66	7.5	4–13.9	<0.001
A allele	23	23	146	73	3.7	2.5–5.6	<0.001

Table 12: Parameters of disease activity and severity in relation to CD4 genotype

	CD4-11743 (n = 150)			CD4-10845 (n = 150)		
	AA (n = 10)	AC (n = 45)	CC (n = 95)	AA (n = 79)	AG (n = 56)	GG (n = 15)
ESR (mm/h)	43.3 ± 21.7	44.8 ± 21.3	48.3 ± 41.1	42.9 ± 26.2	42.5 ± 19.2	52.2 ± 50.1
RF	82.1 ± 88.3	81.6 ± 62.8	68 ± 81.9	114.2 ± 87.3	49.6 ± 39.3[§]	14.7 ± 34.5[§]*
CRP (mg/l)	13.7 ± 10.1	15.9 ± 19.2	14.5 ± 14.5	15.9 ± 14.4	14 ± 8.4	11.6 ± 9.4
No. of swollen joints	4.0 ± 3.2	5 ± 4.4	4.9 ± 4.7	3.8 ± 4.1	4.4 ± 3.9	4.7 ± 4.9
No. of tender joint	10.1 ± 5.5	11.9 ± 6.6	13.8 ± 7.9[#]	8.09 ± 5.9	12.1 ± 5.9[§]	15.8 ± 7.9[§]*
HAQ	1.4 ± 0.9	1.5 ± 0.7	1.1 ± 0.75	1.1 ± 0.8	1.6 ± 0.6[§]	1.6 ± 0.8[§]

[#]Significant difference from AA of CD4-11743,
[§] Significant difference from AAof CD4-10845,
* Significant difference from AG of CD4-10845

V. Discussion

This is the first study to our knowledge conducted in Saudi RA patients to determine the relationship between the occurrence of RA and the presence of the IL-4RαI50V and Q576R genotypes and identify IL-4Rα gene as a predictor for disease progression. Early identification of reliable predictors of severity of the disease is important as the erosions are correlated with clinical outcome, such as disability[39,40].Moreover, O'Dell reported that early treatment may retard bone destruction[41]. Therefore, the potential to early predict the individual who will require more aggressive treatment would be especially valuable.

The higher prevalence of autoimmunity in women suggests that sex hormones might play a role in susceptibility. Estrogen is thought to exert a biphasic dose effect, lower levels enhance while high levels inhibit specific immune activities, whereas progesterone promotes the development of Th2 cells which inhibits the proliferation of the Th1 cells[42]. Pregnancy is associated with a marked increase of estrogen, progesterone and cortisol in the plasma and brings about remission in autoimmune disorders such as RA and multiple sclerosis which are both driven by Th1 cytokines[43].

In RA patients, the frequencies of IV genotype were significantly increased compared to control group. The present study demonstrates that subjects with IV genotype were significantly more likely to have RA. However, Prots et al,did not find any evidence of increased RA risk associated with IV genotype in German[44].

The present study demonstrated there was 2.6-fold increased risk to develop erosive RA in subjects carrying VV genotype. However, subjects carrying QR genotype protected from developing erosive RA. The association of VV genotypes with bone erosions in RA patients suggests the potential role of these polymorphisms to be used as a predictive marker. In fact, the estimated predictive value of the IL4Rα polymorphism revealed the powerful capacity of the VV genotype to identify individuals with a high risk of early bone erosion. Identification of patients homozygous for the V50 allele permitted the prediction of a rapidly erosive disease course.

In accordance with our data, Prots et al, reported an association of IL-4R I50V variant with the rapid development of erosions in German RA patients[44]. Also, Pawlik et al,found that the IL-4R I50V variant has been associated with increased radiological damage during the first 2 years of disease[45], while Marinou et al, failed to support such associations in an English population[46].Burgos et al,also, failed to support such associations in an African American population. Perhaps this effect was not observed due to the different racial/ethnic composition of the patients studied[47].

The association of IL-4R I50V and RA may be explained by recent study of Ford et al, which found a direct effect of the V50 polymorphism in the IL-4Rα as it led to persistence of STAT6 activation in response to IL-4 and persistence activation of a STAT6-dependentgene[48].Since the V50 polymorphism is located in the extracellular domain of the IL-4Rαchain, the prolongation of STAT6 phosphorylationin V50-IL-4Rα was likely not due to modulation of these intracellular mechanisms[48]. The prolongation of STAT6 phosphorylationin V50-IL-4Rαwas dependent upon continued JAK activity[49]. Nevertheless, Ford et al, speculated that the V50 polymorphism may also impact glycosylation of the human IL-4Rαandthat the relative impact may be cell type specific[48]. This glycosylation could influence IL-4 binding parameters leading to alterations in persistence of IL-4 signaling and gene expression in human patients.

IgM RF is present in 60–80% of patients with RA and the titer correlates with disease severity and extra-articular manifestations. Patients with high RF titers have articular damage more persistent and severe than those with low titers or negative RF[50].Numerous patients who eventually develop RA have positive RF and other serological abnormalities many years before development of RA symptoms[51].We found that patients with VV and QQ genotypes show significant increase in the levels of RF, while Moreno et al,reported that R allele was associated with high RF titers in patients with RA[46]. Also, we found that carriers of V allele and Q allele were significantly more likely to develop sever RA. On the contrary, Moreno et al, found an association of II genotypes with the presence of RF in RA patients and with a history of articular joint replacement[46].

SNPs are highly abundant, stable, and distributed throughout the genome. These variations are associated with diversity in the population, individuality, susceptibility to diseases[43], and differential response to medical treatment[52].

Of all the SNPs that affect the TNF gene, the -308 TNF-α promoter polymorphism is probably the most relevant for increased TNF production, thus representing a genetic risk factor for RA predisposition[53].Our results of the -308 TNF-α polymorphism in RA patients and controls demonstrated that the subjects GA genotype were significantly more likely to have susceptibility to RA. Although, these frequency distribution differences remain for the G/A genotype the most significant reside in the A/A genotype, and Cvetkovic et al,who demonstrated that RA patients GA heterozygous for the -308 TNF-α gene polymorphism had a more severe course of disease [55].

Our results of the -308 TNF-α polymorphism in RA patients and controls demonstrated that the TNF-αA alleleis 14.37% and 5% of individuals, respectively. The odds ratio (OR = 1, P = 0.05) suggests an association between the presence of the polymorphism and the disease, also Correa et al,found that the TNF-α -308Aallele was associated with an increased risk of RA[54]. The influence of the TNF-α polymorphism on RA susceptibility or severity may depend on the differences in TNF-α synthesis. The production of TNF-α may be associated with the TNF-α promoter polymorphism. Nevertheless, the association between TNF-α production and the -308 G/A TNF-α promoter polymorphism remains unclear. Functional studies of the -308 G/A TNF-α polymorphism have produced conflicting results, with some investigators reporting higher transcription of the gene in the presence of the A allele, whereas others have not[55].

In the present study, we found a higher serum TNF-α level in the -308GG and -308GA genotype than the -308AA genotypes, this confirmed with Oregon-Romero et al, who found that the -308GG genotype was associated with high TNF-α mRNA levels in RA patients and healthy subjects[56]. This result was not confirmed with other two studies Prasad et al, who reported that -308GA and -308AA genotypes had significantly higher serum levels of TNF-a compared with-308GG genotype[57].Helmig etal, that Gallele of TNF-α-308 is associated with a significantly higher TNF-α mRNA expression compared to the Aallele[58]**.**Identification of other genetic markers associated with susceptibility to or severity of RA is currently an important challenge. Moreover, studied polymorphism may be important in determining treatment responses to new anti-TNF-α therapy[59,60].

It may be speculated that polymorphism within the TNFR genes could alter binding of ligands such as TNF-α or cleavage enzymes, thereby leading to an inappropriate inflammatory response due to excessive circulating TNF-α, and hence contributing to RA susceptibility[61].Our results of the TNFRII polymorphism in RA patients and controls demonstrated that the TNFRII R allele was41.8% and 28.1% of individuals, respectively. The odds ratio (OR = 3.19, P = 0.009) isevaluate the possible association between presence of the TNFRII 196R allele and RA diagnosis and prognosis, this result agree with Goëb et al, studywhich shows that the TNFRII 196R allele appears to be significantly associated with RA.

The results of the present study support the hypothesis that there is an association between the TNFRII -196 M/R gene polymorphism and the functional severity of early RA[32].Recently, TNFRII -196R transfectants have been shown to be associated with higher production of IL-6[16], which plays a crucial role in the pathogenesis of RA. This result support the idea that the genes for susceptibility to systemic lupus erythematosus and RA are partially overlapping[63].The present findings revealed statistical significance between the TNFRII196R allele and RA diagnosis. The frequencies of the TNFRII196R allele observed in the present study are not statistically different from the previously reported frequencies in the UK and the French RA populations[64,65]. In contrary Shibue et al,reported that the TNFRII position 196 polymorphism (TNFRII -196M/R) was not significantly associated with RA in Japanese patients[66].

As previously reported by van der Helm-van Mil[67], we observed a lack of association between the TNFRII 196R allele and the functional severity of RA, which is in disagreement with the findings reported by Constantin et al,[32]. There may be several explanations for these discrepancies, including the heterogeneity of the studied population, differences in the selected outcome criterion between studies, and the influence of treatment.

Most human autoimmune diseases require CD4 T cells for their development including Type 1 diabetes mellitus(T1DM)[68,69], vitiligo[8], and RA which are considered one of the most common autoimmune diseases[70]. RA is assumed to be an autoimmune disease, based on the presence of auto antibodies in the serum and remarkable lymphocyte infiltration into the synovium[65]. Among the infiltrating cells, CD4 T cells are the main cell population and are considered to play a crucial role in the pathogenesis of RA[71]. CD4 is a co receptor that assists the Tcell receptor (TCR) to activate its T cell following an interaction with an antigen-presenting cell. The genetic regulation of theCD4 Tcell activity may influence tolerance or tissue destruction in RA. T cells indirectly mediate autoantibody production, joint inflammation, and bone resorption[72].

In our study, we found that the frequencies of CC genotype were significantly increased in patients with RA compared with control group. However, there were nosignificant differences between CD4-10845 polymorphisms in RA and control group.

In accordance with our data, Sui-Foon et al, found a higher frequency of the CD4-11743C allele and a lower frequency of the CD4 -11743A allele, compared with the controls[38]. In addition, they suggested that CD4-11743C allele increased the risk of development of RA. On the contrary, Sui-Foon et al, found that patients with RA had a significantly higher frequency of the CD4-10845 AA genotype compared with the controls[73]. Similarly, further comparison of the allele frequencies between patients with RA and controls are made and they revealed that the former had a higher frequency of the A allele and a lower frequency of the G allele. Interestingly, they added that CD4-10845 AA genotype and CD4-10845A allele increased the risk of RA development.

Genetic polymorphisms at the CD4 enhancer locus have been shown to be associated with the risk of development of RA and systemic lupuserythematosus[38]. The molecular mechanisms controlling CD4 gene expression are very complex and are controlled by many different signals beside CD4 enhancer as the thymocyte develops which need more in-depth studies concerning both CD4enhancer and CD4 silencer[9].

VI. Conclusion

Our findings provided the evidence that IL-4RαI50V polymorphism was associated with RA susceptibility in Saudi women. IL-4RαI50V and IL-4RαQ576R polymorphisms may be helpful in early detection of erosive RA in Saudi women.

We found a positive association of the A allele of the −308 promoter polymorphism of the TNF-α gene with more susceptibility of RA disease, also subjects GA genotype have high TNF-α serum level compared with AA genotype. Our findings suggested thatTNFRII -196R allele may be associated with RA diagnosis, also the 308GA genotype of TNF-**α**was associated with a significantly higher serum TNF-αlevel compared to the -308GG genotype, also there was an association of the A allele of the -308 promoter polymorphism with RA susceptibility. Genetic polymorphisms at the CD4 enhancer gene are one of important factors which associated with susceptibility and severity of RA and can serve as a genetic marker for the risk of development of RA. Similar studies in other populations and with larger sample size are needed. Also, functional studies on the role and function of the polymorphism of CD4 enhancer and CD4 pentanucleotide repeat, silencer, and other disputable cis-acting elements that affect CD4 gene expression will be also necessary.

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