Analysis of Association between Interleukin-6 (*IL6*), Interleukin-13 (*IL*13) and Tumor Necrosis Factor-Alpha (*TNF-alpha*) Gene Polymorphisms and Genetic Susceptibility of Rheumatoid Arthritis in Kuwaiti Arab

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Abstract

Background: Rheumatoid arthritis (RA) is a common autoimmune disease in which a combination of risk alleles from different susceptibility genes predisposes the patients to develop clinical symptoms following exposure to environmental factors. RA is a chronic and progressive disease characterized by synovial inflammation that results in destruction in the affected joints and severe problems in individual's mobility. Several immune-related risk factors have been associated with RA, these include single nucleotide polymorphisms (SNPs) in cytokine genes. The impact of these cytokine gene polymorphisms is due to their association either with elevated serum levels and/or variations in their serum levels are associated with disease-onset and progression. The objective of this study was to investigate the role of Interleukin-6 (*IL6*), Interleukin-13 (*IL*13) and Tumor necrosis factor-alpha (*TNF-alpha*) gene polymorphisms in genetic susceptibility of RA in Kuwaiti patients. **Methods:** We have determined the genotypes of *IL6* gene (−174G/C; rs1800795), *IL*13 gene (R130Q; rs20541) and *TNF-alpha* gene (−308A/G' rs1800629) polymorphisms in 192 Kuwaiti patients with RA and compared it to that in 104 healthy controls. The diagnosis of RA was based on the American College of Rheumatology (ACR) classification criteria. The genotypes for *IL6*, *IL*13 and *TNF-alpha* gene polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methods and confirmed by DNA sequencing. **Results:** The frequency of *IL6* gene (−174G/C;

rs1800795) and *TNF-alpha* gene (−308A/G' rs1800629) polymorphisms manifested a statistically significant difference between Kuwaiti RA patients and controls (P = 0.02 and 0.002 respectively). In contrast, the frequency of *IL*13 gene (R130Q; rs20541) polymorphism did not show a significant difference between Kuwaiti RA patients and controls. **Conclusions:** Our data showed an association of two cytokine gene polymorphisms (*i.e. IL*6 gene −174G/C; rs1800795 polymorphism, and *TNF-alpha* gene −308A/G, rs1800629 polymorphism) with RA in Kuwaiti patients highlighting their significant contribution in genetic susceptibility of this chronic disease possibly along with other factors.

Keywords

Genotype, Cytokine Gene, Polymorphism, Rheumatoid Arthritis, Kuwait

1. Introduction

Rheumatoid arthritis (RA, OMIM# 180300) is a common chronic, systemic inflammatory disease with autoimmune etiology and affects the joints. It has a prevalence of 0.1% - 0.5% in people of Asian descent and 0.3% - 1.1% in populations with European ancestry [1]. RA is characterized by inflammation of the synovial joint tissues and formation of the rheumatoid pannus, which damages the adjacent cartilage and bone and results in joint deformity [2]. RA is a complex, multifactorial disease which is thought to manifest itself as a result of interaction between environmental and genetic factors [3] [4]. The circulating autoantibodies, specifically, rheumatoid factor (RF) and anti-citrulinated protein/peptide antibodies (ACPA) have been implicated in the pathogenesis of RA.

Proinflammatory cytokines play key roles in the inflammation process and synovial cell proliferation which is associated with joint destruction in RA patients [5]. It has been reported that, in RA, there is an imbalance in the production of cytokines with a relative excess of proinflammatory molecules including interleukin-6 (*IL*6) and tumor necrosis factor (TNF) compared with the anti-inflammatory mediators such as *IL*10 and *IL*13 [6]. It has been shown that *IL*6 is expressed at high levels in RA patients [7]. Previous reports suggest that this crucial cytokine is a key mediator of systemic and local inflammation in RA patients [6] [7]. It has also been demonstrated that *IL*6 is directly implicated in the production of serum C-reactive protein (CRP) and a significant correlation exists between CRP levels at the time of disease diagnosis and the presence of endothelial dysfunction in patients with psoriatic arthritis [8]. The elevated serum *IL*6 levels in RA patients have also been shown to correlate with disease activity [9] and radiologic joint damage [10]. A functional polymorphism in the 5'-flanking region of the *IL*6 gene has been shown to determine differences in the control of *IL*6 expression [11]. This polymorphism, which results in a single nucleotide change from G to C, at position −174 of the *IL*6 gene, affects the gene transcrip-

tion rate and has been shown to be associated with different plasma levels of *IL*6 [11]. Interestingly, *IL*6 plasma levels in individuals carrying the *IL*6 GG genotype were found to be twice as high as those detected in individuals with a homozygous CC genotype [11].

Interleukin-13 (*IL*13) is an anti-inflammatory cytokine which is produced mainly by CD4+ T cells with Th2 features and has been shown to play an important role in Th2-mediated diseases [12]. High levels of *IL*13 have been found in synovial fluid from actively inflamed joints of RA patients [13]. The role of *IL*13 in pathogenesis of RA is not clear but it has been postulated that it may downgrade the inflammatory process by suppressing the production of Th1 cytokines [14]. The human *IL*13 gene is located on chromosome 5q31. A polymorphism in the *IL*13 gene (R130Q; rs20541), has been associated with some autoimmune diseases in Caucasians [15].

TNF-alpha is a pro-inflammatory cytokine that has been shown to play a key role in the pathology of autoimmune and inflammatory diseases [16]. *TNF-alpha* gene carries the (−308A/G' rs1800629) single nucleotide polymorphism (SNP), which has been associated with inflammatory diseases including psoriatic arthritis [16]. The (−308A/G' rs1800629) promoter polymorphism in the *TNF-alpha* gene has also been shown to be associated with differential response to *TNF-alpha* blocking therapy in RA patients [17]. It has been reported that patients carrying the −308A allele express higher levels of serum *TNF-alpha* [18] and that patients with a high response to TNF-blockers could also have higher *TNF-alpha* bioactivity [19] while the poorer responders are those who produce lower level of *TNF-alpha* [17]. In one previous report, it was shown that the RA patients with *TNF-alpha* −308GG genotype, responded better to TNF-blockers at week 24 [20].

The outcomes are difficult to predict in RA patients as it is a highly heterogeneous disease. Also, the extent of joint damage in patients with RA varies considerably among individuals. Patients with more rapid progression need more extensive therapy such as early treatment with biologic agents, than patients with slower progressive disease. The heterogeneity of RA can be explained, at least in part, by the genetic factors [2], however, important question is that whether an individual's response to therapy can be predicted based on the genetic factors. The development of biologic agents such as tumor necrosis factor (TNF) inhibitors has revolutionized the treatment of RA [2]. We have undertaken this case-control design study to investigate the involvement of three cytokine gene polymorphisms, namely the *IL*6 gene (−174G/C; rs1800795), *IL*13 gene (R130Q; rs20541), and the *TNF-alpha* gene (−308A/G'; rs1800629) polymorphism in genetic susceptibility to RA in Kuwaiti Arab patients.

2. Patients and Methods

This study included 192 Kuwaiti Arab patients with rheumatoid arthritis (RA) and 104 healthy controls with matched ethnicity. The RA patients were seen at

the Rheumatic Disease Unit, Amiri Hospital, Kuwait over a period of two years (2019-2021). The RA patients fulfilled the American College of Rheumatology (ACR) classification criteria for RA [21]. A complete clinical assessment was carried out for RA patient. Standardized joint counts and patients' ratings of general health on a 100 mm visual analog scale were recorded. The data was collected and stored in a specifically designed form. The four-variable Disease Activity Score 28 (DAS28-CRP) was computed by the formula described earlier [22]. To define remission, a cutoff point of <2.6 was used for the DAS28-CRP, and it corresponded with the modified American Rheumatism Association criteria for remission [23] [24]. The patients were characterized, on the basis of disease duration, into early (<3 months), established (>3 months to up to 2 years), or late (>2 years) disease [25] [26]. These clinical subdivisions of the RA patients corresponded with the maximum number of joints involved at any stage into mild (3 - 6 joints), moderate (>6 but <20 Joints) or severe disease (>20 joints) respectively [27] [28]. Radiological investigations of hands, wrists and feet were performed and read by a radiologist to assess RA erosions. The strategy for treatment of the RA patients was based on published guidelines [29]. The RA patients received treatment with Disease Modifying Anti-Rheumatic Drugs (DMARDS) and/or biologicals, and when appropriate, Non-Steroidal Anti-Inflammatory Drugs (NSAID) and/or corticosteroids were also used. The control subjects were healthy Kuwaiti nationals and were evaluated by a trained Rheumatologist for their health status. Anyone with suspected autoimmune, bone and/or joint diseases in the families were excluded from the list of controls.

Determination of the genotypes

Blood samples (approximately 5 mL) were collected from RA patients and controls by a trained technologist under appropriate conditions and anticoagulated by EDTA. DNA was isolated from the peripheral leukocytes using a standard method [30].

IL-6 gene (−174G/C; rs1800795) polymorphism

The genotypes for *IL*6 gene (−174G/C; rs1800795) polymorphism were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method described earlier [31], using the primers listed below:

Forward primer: 5'-GGAGTCACACACTCCACCT-3'

Reverse primer: 5'-GTGGGGCTGATTGGAAACC-3'

The PCR reactions were carried out in a total volume of 25 μL containing 100 ng of genomic DNA, 10 pmoles of each primer, 2 mM $MgCl_2$, 0.2 mM deoxynucleotides (dNTPs), 1 × buffer, and 2U of Taq DNA polymerase. For *IL*-6 gene (−174G/C) polymorphism, the amplification was performed for 35 cycles with an initial denaturation step at 94˚C for 5 min followed by the PCR cycles of 94˚C for 1 min, annealing at 65˚C for 1 min, 72˚C for 1 min and then an extension step at 72˚C for 10 min. The polymorphism was identified by cleavage of the PCR products with restriction enzyme *Sfa*N1. The G → C change at position 174 created a restriction site for this enzyme and as a consequence G-allele pro-

duced an un-cleaved 532 bp product while the C-allele yielded two products of 474 and 58 bp respectively upon digestion of the PCR products with *Sfa*N1.The DNA cleavage fragments were resolved by electrophoresis on a 3% agarose gel and were visualized under UV light after Ethidium bromide staining.

IL-13 gene polymorphism (R130Q; rs20541)

The *IL*13 gene polymorphism (R130Q; rs20541) genotypes were determined by PCR-RFLP methods described earlier by [32] [33]. The PCR primer sequences used were:

Sense primer: 5'-CTTCCGTGAGGACTGAATGAGACGGTC-3'

Antisense primer: 5'-GCAAATAATGATGCTTTCGAAGTTTCAGTGGA-3'

For *IL*13 gene (R130Q; rs20541) polymorphism, the amplification was carried out at 94˚C for 5 minutes followed by 35 cycles of 94˚C for 45 seconds, 67˚C for 45 seconds and 72˚C for 30 seconds followed by an extension at 72˚C for 5 min. The PCR products were cleaved with restriction enzyme *Nla*IV (0.5 U) at 37˚C for 3 hours and then analyzed by agarose gel electrophoresis as described earlier. The expected products were 210 bp and 26 bp when a normal R130-genotype (QQ) was present and in the case of mutant −130Q (RR) genotype, the expected product sizes were 178 bp, 32 and 26 bp respectively.

TNF-alpha gene (−308A/G; rs1800629) polymorphism

The genotypes for *TNF-alpha* gene (−308A/G' rs1800629) polymorphism were determined by PCR-RFLP method using the primers listed below and described earlier [34]:

Forward primer: 5'-AGGCAATAGGTTTTGAGGGCCAT-3'

Reverse primer: 5'-TCCTCCCTGCTCCGATTCCG-3'

The PCR reactions were carried out in a total volume of 25 µL containing 100 ng of genomic DNA, 10 pmoles of each primer, 2 mM $MgCl_2$, 0.2 mM deoxynucleotides (dNTPs), 1 × buffer, and 2U of Taq polymerase. The amplification was performed by incubation at 94˚C for 5 min, followed by 35 cycles, each with 94˚C for 1 min, annealing at 60˚C for 1 min, 72˚C for 1 min, followed by an extension step at 72˚C for 10 min. The polymorphism was identified by *Nco*1 restriction endonuclease digestion of the PCR-amplified fragment at 37˚C for 1 hour. The expected product sizes were 107 bp in the case of A-allele and 87 and 20 bp in the G-allele. The products of restriction enzyme cleavage were analyzed by electrophoresis on a 3% agarose gel and were visualized by UV light after Ethidium bromide staining.

The genotypes were confirmed by determining the sequence of PCR products using ABI 3130 genetic analyzer.

Statistical analysis

The data was analyzed using the Statistical Package for the Social Sciences version 23 (SPSS, Chicago *IL*, USA). The sample size was calculated by using the Fisher's formula: (https://www.geopoll.com/blog/sample-size-research/). The frequencies of various genotypes and alleles detected among RA patients and controls were calculated by direct counting. The confidence interval (CI) was set at 95% and statistical significance was set at $P < 0.05$ (two-tailed). Fisher's Exact

test was used to determine statistical significance of the differences between genotype and allele frequency in the RA patients and controls. For calculation of the statistical significance in co-dominant and dominant genetic models, the genotype frequency in homozygous GG, RR and AA subjects and the allele frequency of "G/R/A" were considered as reference (as described previously, [31] [32] [33] [34]). In the case of dominant model, the genotype frequencies in respective heterozygous and homozygous subjects were pooled and then analyzed. A posteriori power analysis was carried out to evaluate strength of the statistical analysis. The genotype distribution was tested for Hardy Weinberg equilibrium by the goodness of fit method using MSTAT software (version 7.0.1). Kuwait is a small country with a population of nearly four million and the rate of consanguinity is high (nearly 54%); this may cause type 2 errors in data analysis.

3. Results

The RA patients' group (total 192) consisted of 165 females and 27 males, with female to male ratio of 7:1. The mean age of Kuwaiti RA patients was 54.65 years ± 13.16 and mean duration of disease was 9 years (±7). In 149/167 (89%) RA patients, the disease was classified as seropositive, while 18 RA patients (11%) were seronegative (some clinical data was not available from 25 RA patients). The evaluation of joints by radiography revealed erosive disease in 31/167 (19%) RA patients whereas 136 (81%) patients had no evidence of bone erosions. Only 8/149 (5%) RA patients had extra-articular manifestations and in 141 patients (95%) the RA was limited to the joints. In patients who manifested extra-articular manifestations, 6/8 had Sicca/Sjogren syndrome and one had autoimmune uveitis. The family history of the disease was noted in 52/192 (27%) RA patients who had one or more immediate members of their family affected with RA.

The frequencies of *IL*6 gene (−174G/C; rs1800795) polymorphism genotypes and alleles have been presented in Table 1. The frequency of homozygous CC genotype was considerably higher in the RA patients compared to that in the controls (67.2% vs. 48.1%; OR, 1.20; P = 0.04; Table 1). Similarly, the frequency of "C" allele of the *IL*6 gene (−174G/C; rs1800795) polymorphism also showed a statistically significant difference between RA patients and the controls in the co-dominant model of genetic analysis (80% vs. 71%; OR 1.59; P = 0.02, Table 1).

The frequency of *IL*13 gene (R130Q; rs20541) polymorphism genotypes and alleles have been presented in Table 2. No significant difference was detected in the genotype frequency of *IL*13 gene polymorphism between RA patients and controls in both the co-dominant and dominant models of genetic analysis (Table 2). The frequency of 'Q' allele of the *IL*13 gene (R130Q; rs20541) polymorphism also did not show a significant difference between RA patients and controls (Table 2).

The genotype and allele frequencies of *TNF-alpha* gene (−308A/G; rs1800629) polymorphism have been presented in Table 3. A statistically significant difference was detected in the frequency of homozygous GG genotype between RA patients and controls in the co-dominant model of genetic analysis (77.1% vs.

45.2%; OR, 1.30; P = 0.03; Table 3). The frequency of "C" allele of the *TNF-alpha* gene (−308A/G; rs1800629) polymorphism was also significantly higher in RA patients compared to that in the controls (80% vs. 68.3%; OR, 1.85; P = 0.002; Table 3).

Table 1. Frequency of *IL*6 gene (−174G/C; rs1800795) polymorphism genotypes and alleles in Kuwaiti PsA patients and controls.

Genotype/Alleles	Patients N = 192 (%)	Controls N = 104 (%)	OR (95% CI*)	P-value**
Co-dominant				
GG	15 (7.8)	6 (5.8)	1.00 (Reference)[a]	
GC	48 (25.0)	48 (46.2)	0.4 (0.14 -1.12)	0.09
CC	129 (67.2)	50 (48.1)	1.20 (0.38 - 2.81)	**0.04**
Dominant				
GG	15 (7.8)	6 (5.8)	1.00 (Reference)[a]	
GC/CC (GC+CC)	177 (92.2)	98 (94.2)	0.72 (0.27 - 1.92)	0.6
Alleles	**N = 384 (%)**	**N = 208 (%)**		
G	78 (20.3)	60 (28.9)	1.00 (Reference)[a]	
C	306 (80.0)	148 (71.0)	1.59 (1.10 - 2.35)	**0.02**

*OR (95% CI), odds ratio at 95% confidence interval; **P-values were considered significant when < 0.05 (shown in bold). [a]Genotype frequency in homozygous subjects with GG genotype and allele frequency of "G" were considered as reference for calculation of statistical significance using Fisher's Exact test.

Table 2. Frequency of *IL*13 gene polymorphism (R130Q; rs20541) genotypes and alleles in Kuwaiti PsA patients and controls.

Genotype/Alleles	Patients N = 192 (%)	Controls N = 104 (%)	OR (95% CI*)	P-value**
Co-dominant				
RR	7 (3.6)	3 (2.9)	1.00 (Reference)[a]	
RQ	43 (22.4)	19 (18.3)	0.97 (0.23 - 4.16)	1.00
QQ	142 (74.0)	82 (78.9)	0.74 (0.19 - 2.95)	0.75
Dominant				
RR	7 (3.6)	3 (2.9)	1.00 (Reference)[a]	
RQ/QQ (RQ+QQ)	185 (96.4)	101 (97.1)	0.78 (020 - 3.10)	0.9
Alleles	**N = 384 (%)**	**N = 208 (%)**		
R	57 (14.8)	25 (12.0)	1.00 (Reference)[a]	
Q	327 (85.2)	183 (88.0)	0.78 (0.47 - 1.30)	0.38

*OR (95% CI), odds ratio at 95% confidence interval; **P-values were considered significant when < 0.05. [a]Genotype frequency in homozygous RR subjects and allele frequency of "R" were considered as reference for calculation of statistical significance using Fisher's Exact test.

Table 3. Frequency of *TNF-alpha* gene (−308A/G; rs1800629) polymorphism genotypes and alleles in Kuwaiti PsA patients and controls.

Genotype/Alleles	Patients N =192 (%)	Controls N = 104 (%)	OR (95% CI*)	P-value**
Co-dominant				
AA	33 (17.2)	9 (8.7)	1.00 (Reference)[a]	
AG	11 (5.7)	48 (46.2)	0.07 (0.03 - 0.17)	<0.0001
GG	148 (77.1)	47 (45.2)	1.30 (0.38 - 1.93)	0.03
Dominant				
AA	33 (17.2)	9 (8.7)	1.00 (Reference)[a]	
AG/GG (AG+GG)	159 (77.8)	95 (91.4)	1.46 (0.21 - 1.80)	0.05
Alleles	N = 384 (%)	N = 208 (%)		
A	77 (20.05)	66 (31.7)	1.00 (Reference)[a]	
G	307 (80.0)	142 (68.3)	1.85 (1.26 - 2.72)	0.002

*OR (95% CI), odds ratio at 95% confidence interval; **P-values were considered significant when < 0.05 (shown in bold). [a]Genotype frequency in homozygous AA subjects and allele frequency of "A" were considered as reference for calculation of statistical significance using Fisher's Exact test.

4. Discussion

*IL*6 is a pro-inflammatory cytokine and has been shown to be a mediator of the acute phase response and its elevated levels have been reported in synovial fluid and tissue of RA patients [35]. The role of *IL*6 in the pathology of RA is further supported by the evidence that blockade of *IL*6 activity with a soluble anti-*IL*6 molecule Tocilizumab reduced disease activity and its radiological progression [36]. Our results showed a statistically significant association between the CC genotype and "C" allele of the *IL*6 gene (−174G/C; rs1800795) polymorphism and RA in Kuwaiti patients (Table 1). Similar results have been reported from Egypt in which "C" allele was associated with RA susceptibility [37]. This is particularly interesting because it has been shown that the RA patients from Poland, who have homozygous CC genotype are characterized with higher average *IL*6 serum levels before anti-TNF treatment as compared to the patient carrying the "C" allele [38]. Similar findings have been reported from Egypt, which showed higher serum concentration of *IL*6 in RA compared to the controls [39] [40]. A study in European RA patients also reported higher serum levels of *IL*6 associated with "C" allele of the *IL*6 gene polymorphism [41]. A previous report from Brazil showed that the higher *IL*6 serum levels were associated with disease severity measured by CDAI (clinical disease activity index), DAS 28 scores, appearance of bone erosions and ESR levels [42]. Our results and those reported from Egypt [37] are in sharp contrast to an earlier report from U.K. in which the GG genotype has been shown to be associated with the disease onset in RA patients [35]. Similar findings were reported in a study in juvenile idiopathic

arthritis (JIA) where a positive association with GG genotype of the *IL*6 gene (−174G/A) polymorphism was observed [11]. A study from Chinese Han population of RA patients showed that variant polymorphism of *IL*6 gene played a major role in susceptibility to RA [43]. Although these authors reported that this association was only nominally significant in their RA patients, however, they found a stronger association when all previous studies were synthesized together with their data in a meta-analysis [43]. Two association studies of *IL*6 gene (−174G/C; rs1800795) polymorphism with RA have been reported from RA patients from Poland [38] [44]. In both these reports, no significant differences in genotype and allele distribution were detected between the Polish RA patients and the controls; the heterozygous GC was found to be the most frequent genotype [38] [44]. Although a report from northwestern Spain did not find an association of *IL*6 gene (−174G/C; rs1800795) polymorphism with RA susceptibility, but its association was reported with sub-clinical atherosclerosis manifested by severe endothelial dysfunction [5]. A study from Serbia reported no differences between genotypic groups of *IL*6 gene (−174G/C; rs1800795) polymorphism either in disease onset or in disease duration [45]. This study from Serbia also reported that *IL*6 levels in RA patients were significantly reduced following etanercept treatment and suggested that increased expression of *IL*6 in "C" allele carriers (either homozygous or heterozygous) can result in increased inflammatory joint activity, and poor response to anti-TNF therapy. They further reported that a significantly lower percentage of DAS28 responders in the "C" allele carriers (CC or GC genotypes) compared to GG homozygotes after 12 months of treatment. These findings therefore suggest that *IL*6 gene (−174G/C; rs1800795) polymorphism genotypes can be used to predict the outcome of suppressive treatment (CC or GC genotypes would be associated with the worst outcome) [45]. In the light of these findings, our data from Kuwaiti Arab patients with rheumatoid arthritis demonstrate that majority (patients with CC and GC genotypes, 92.2%) would likely be poor responders to suppressive treatment thus highlighting the importance of this polymorphism as a prognostic marker for predicting the treatment outcome.

The results on the genotype and allele frequency of *IL*13 gene (R130Q; rs20541) polymorphism did not show a significant difference between Kuwaiti PsA patients and the controls (Table 2) indicating that on its own, this polymorphism may not be considered as a susceptibility locus for RA in Kuwaiti population. This is perhaps not surprising because it has been shown that *IL*13 is an anti-inflammatory cytokine whereas RA, is a disease with autoimmune and inflammatory pathology. It has also been reported that *IL*13 is predominantly produced by CD+ T cells with Th2 characteristics and plays a role in Th2 mediated diseases possibly by affecting the Th1-Th2 balance [46]. The lack of association between this polymorphism in Kuwaiti RA patients shows that it may not have a significant direct role in manifesting the disease symptoms, although an indirect effect via immune system modulation cannot be completely ruled out.

The most important finding in this study is that a statistically significant difference was detected in the frequency of GG genotype and "G" allele of the *TNF-alpha* gene (−308A/G, rs1800629) polymorphism between Kuwaiti RA patients and controls (Table 3). The first biological drugs registered for RA therapy were *TNF-alpha* inhibitors, however, their cost remain very high. Although the therapy with *TNF-alpha* inhibitors constituted a breakthrough in RA treatment, no improvement is still achieved in 30% cases and another 20% patients discontinue treatment due to side effects [17]. The search for suitable markers which would allow prediction of a good response to therapy with biologicals, including *TNF-alpha* inhibitors is ongoing. It has been suggested that variations in the genes implicated in *TNF-alpha* pathway can potentially influence the outcome of anti-TNF therapy [17]. The pharmacogenomic studies on the influence of *TNF-alpha* gene polymorphisms on response to TNF blocking treatment have reported conflicting results [17]. It has been shown that RA patients who carry the −308A allele express higher levels of *TNF-alpha* [18] and that the patients with a high response to TNF blockers could also have a high *TNF-alpha* bioactivity or high synovial level of this cytokine at the baseline [19]. In this context, poor responders are expected to have lower levels of *TNF-alpha*. Two previous studies reported an association of the *TNF-alpha* −308GG genotype with better response to anti-TNF treatment with infliximab (INF) [47] or etanercept (ETA) [48]. In another study, the TNF-308GG genotype was found to be associated with a better response to TNF blockers at week 24 [20]. In the light of these reports, it can be anticipated that in Kuwaiti RA patients, in which the majority (82.2%) had GG or AG genotypes of the *TNF-alpha* gene (−308A/G, rs1800629) polymorphism, can be expected to be 'better responders' to the TNF-blocking therapy. This highlights the role of *TNF-alpha* gene (−308A/G, rs1800629) polymorphism not only in RA susceptibility in Kuwaiti Arab patients with rheumatoid arthritis but possibly also in monitoring response to the anti-TNF therapy further highlighting their role as prognostic markers.

5. Conclusion

Our data showed an association of two cytokine gene polymorphisms (*i.e. IL6* gene −174G/C; rs1800795 polymorphism, and *TNF-alpha* gene −308A/G, rs1800629 polymorphism) with RA in Kuwaiti patients highlighting their significant contribution in genetic susceptibility of this chronic disease possibly along with other factors.

Ethical Approval and Consent

The study was carried out strictly in accordance with the Helsinki-II Declaration. The Ethics Committee of Health Sciences Centre, Kuwait University approved this study (Ref. No. VDR/EC/3243). Written informed consent was obtained from all the study subjects.

Acknowledgements

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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List of abbreviations

RA   rheumatoid arthritis

IL   interleukin

PCR   polymerase chain reaction

RFLP   restriction fragment length polymorphism

INF   infliximab

ETA   etanercept

RF   rheumatoid factor

SNP   single nucleotide polymorphism

UV   ultra-violet

bp   base pair

CI   confidence interval

OR   odds ratio