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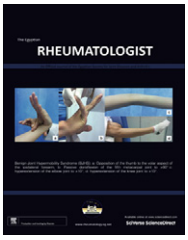

ORIGINAL ARTICLE

Clinical significance of serum interleukin-6 and −174 G/C promoter polymorphism in Rheumatoid arthritis patients

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KEYWORDS

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(−174 G/C) Promoter polymorphism;
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Abstract *Aim of the work:* To evaluate the clinical significance of serum levels of interleukin-6 (IL-6) and −174 G/C promoter polymorphism in Rheumatoid arthritis (RA) patients.

Patients and methods: We studied 37 RA patients and 10 age and gender matched healthy controls. Demographic, clinical and serological data were prospectively evaluated. Disease activity score (DAS28) and Health Assessment Questionnaire (HAQ) were assessed. Serum IL-6 level was measured and promoter (−174G/C) genotyped.

Results: Serum IL-6 levels were significantly higher in RA patients compared to control ($p = 0.04$), especially those with CC promoter polymorphism. Twenty-four patients had GG IL-6 (−174 G/C) gene promoter polymorphism, 11 were GC and 2 CC. Nine controls were GG and 1 GC. In patients with more advanced polymorphism (−174 CC) there was a significantly increased functional impairment (HAQ score) ($p = 0.029$) and platelet count ($p = 0.049$). In those with GG genotype, there was a significant correlation between IL-6 and Morning stiffness duration ($r = 0.44$,$p = 0.03$), while those with GC genotype had a significant negative correlation of the IL-6 level with the parameters of disease activity and the DAS28 ($r = -0.69$,$p = 0.019$). None of the studied parameters would predict the IL-6 promoter polymorphism.

Conclusion: Serum IL-6 levels and −174 G/C promoter polymorphism were higher in RA patients than in healthy controls. The inverse relation of IL-6 with the DAS28 in those with an

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increased IL-6 promoter polymorphism may confirm its increased involvement in the pathogenesis of RA and in the increased disease activity which may point to the need for considering of anti-IL-6 agents in their management plan.

1. Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory, multi-factorial disease sustained by environmental and genetic factors. These seem to be necessary but not sufficient in the disease development, nonetheless they can be responsible of different clinical pictures and response to therapy, and represent potential therapeutic targets. Several genes have been indicated so far in the pathogenesis of RA [1]. Substantial evidence has implicated interleukin-6 (IL-6) in the pathogenesis of RA [2]. The role of the variability of IL-6 production in the pathogenesis and severity of RA is genetically determined [3] and its −174 promoter polymorphism is associated with disease susceptibility and activity and forms a genetic risk factor [4].

Interleukin-6 (IL-6) is a cytokine that can facilitate autoimmune phenomena, amplify acute inflammation and promote the evolution into a chronic inflammatory state. In addition, IL-6 has a pivotal role in synovitis, bone erosions and in the systemic features of inflammation [5]. The transition from acute to chronic inflammation is characterized by a change in cellular infiltrate from predominantly polymorphonuclear neutrophils to mononuclear cells, and IL-6 is a key regulator of this process [2]. Its serum concentrations are significantly elevated in RA patients and they decreased with medical treatment proposing a potential role for IL-6 family cytokines in the pathogenesis of RA [6]. Being a key cytokine for B cell survival and proliferation, IL-6 (−174) promoter polymorphism was identified as a genetic biomarker of response to biologics in RA [7]. The IL-6 system plays a sensitive role in local inflammatory reactions by amplifying leukocyte recruitment and has the capacity to protect against lipopolysaccharide [8]. A role for functional polymorphism and genetically determined lower soluble IL-6 receptor (IL-6R) levels has been suggested as a risk factor for RA [9].

Gene polymorphisms such as those located at Tumor Necrosis Factor (TNF), IL-1 and IL-6 seem to be responsible for more aggressive disease phenotype. The efforts in the post-genomic era can bring to an estimation of the real likelihood of the genetic effect [1]. The −174 C allele confers a higher mortality and plasma level of IL-6 in patients with abdominal aortic aneurysm [10] and a significantly higher relative risk of coronary heart disease in healthy subjects [11]. In a study on Egyptian patients with pemphigus, the IL-6 −174 CC genotype has been reported to represent a marker of increased susceptibility while the GG genotype can be considered a low-risk genotype [12]. Rheumatoid patients homozygous for the IL6 −174 GG genotype had more severe endothelial dysfunction than those carrying the GC or CC genotypes [13]. Given the pleiotropic function of IL-6 it can be anticipated that other inflammatory diseases and bone metabolic conditions might benefit from selective IL-6 signaling inhibition [5]. Despite the important physiological activities of IL-6, dysregulated overproduction is pathologically involved in various immune-mediated inflammatory diseases other than RA, such as systemic lupus erythematosus, adult-onset Still disease, Takayasu arthritis, polyarteritis nodosa, systemic sclerosis, reactive arthritis, dermatomyositis and polymyositis [14].

By virtue of its multiple effects, IL-6 is involved in the various phases of RA development, including the acute phase, immuno-inflammatory phase, and destructive phase. IL-6 has an impact on the many pathogenic factors identified in RA and, consequently, holds promise for targeted treatments and monoclonal antibody (tocilizumab) to transmembrane and soluble IL-6Rα has been found effective. Tocilizumab is now indicated for the treatment of adults with RA who have failed at least one synthetic disease-modifying antirheumatic drug or TNFα antagonist [15]. The discovery of new genes associated with the disease can be relevant in finding potential biomarkers, potentially useful in disease diagnosis and treatment [1]. The clinical efficacy of IL-6 inhibition also underlines the important contribution of this cytokine in RA [16].

The aim of the present study was to evaluate the clinical significance of serum levels of Interleukin-6 (IL-6) and −174 G/C promoter polymorphism in Rheumatoid arthritis (RA) patients and find any association to the clinical and laboratory features and disease activity.

2. Patients and methods

2.1. Study design

Thirty-seven patients (35 females and 2 males) with definite RA diagnosed according to the 2010 ACR/EULAR RA classification criteria [17] were consecutively recruited from the Rheumatology outpatient clinic and department of Cairo University Hospitals. Full history taking and thorough clinical examination were performed for all the patients. Laboratory investigations in the form of complete blood count (CBC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) and plain X-ray of the affected joints were assessed. All patients were regularly receiving methotrexate with or without corticosteroids and chloroquine. Disease activity score (DAS-28) was calculated [18]. The Health Assessment Questionnaire-II (HAQ-II) was used [19]. Radiological grading of the hands and feet of the studied RA patients was assessed and percentage damage was calculated according to the modified Larsen score [20]. Informed consents were taken from the patients and the study was approved by the local ethics committee.

2.2. Methods

2.2.1. Determination of serum IL-6 level

Serum IL-6 was assayed using Human IL-6 ELISA kit (quantitative sandwich enzyme immunoassay technique), provided by Ray Biotech, Inc. (www.raybiotech.com).

2.2.2. Determination of IL-6 gene (−174) promoter polymorphism

Promoter region polymorphism of IL-6 gene (−174 G/C) was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. DNA isolation and IL-6 genotyping: genomic DNA was isolated from white blood cells (WBCs) of peripheral blood using Biospin Whole Blood Genomic DNA Extraction Kit (Bioflux Corporation, Arrow Plaza, Tokyo, Japan).Enzymatic amplification was performed by PCR using Master Taq polymerase enzyme and Hybrid thermal cycler (Promega Corporation, 2800 Woods Hollow Road Madison, W 153711–5399 USA). Amplification of the promoter region (−174G/C) of the IL-6 gene was done as proposed by Pola et al. [21] using 2 primers (were purchased from Operon Biotechnologies (GmbH/Biocampus, Germany). Forward Primer: 5′- GCC TCA ATG ACG ACC TAA GC-3′, and Reverse Primer: 5′- TCA TGG GAA AAT CCC ACA TT-3′. The PCR reaction mixture (25 µl) contained 12.5 µl 2× PCR Master Mix {10 × PCR buffer, 4 mM MgCl2, 0.5 Taq DNA polymerase/µl, 0.4 mM dNTPs (dATP, dCTP, dGTP, dTTP)}, 1 µl of each primer (25 pmol), 3 µl of genomic DNA and 7.5 µl sterilized nuclease-free water. The reaction was carried out with the following cycles: 95 °C for 5 min; 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 61 °C and 30 s extension at 72 °C and a 10-min final extension at 72 °C after completion of the cycles. Then amplified products were digested with 5 units of Fast Digest NLa III restriction enzyme at 37 °C for 10 min (supplied by Fermentas, LT-02241 Vilnius, Lithuania). The digested products were then detected in 3.5% agarose gel containing ethidium bromide by performing E/P on the gel electrophoresis apparatus and visualized by UV transillumination (Promega, USA). A single band at 163 bp identified GG homozygous individuals, two bands at 111 and 52 bp identified CC homozygous individuals, and three bands at 163, 111 and 52 bp indicated a GC heterozygote.

Statistical analysis: The data were collected, tabulated and analyzed by SPSS package version 15 (SPSS corporation, USA). Data were summarized as mean ± SD. Mann–Whitney tests were used for comparative analysis of 2 quantitative data. Non-parametric analysis of variance (Kruskall–Wallis) was used for comparison of more than two groups; Spearman's correlation analysis was used for detection of the relation between 2 variables. Logistic regression analysis was applied to detect the predictors for the elevated IL-6 level and promoter polymorphism. Results were considered significant at $p < 0.05$.

3. Results

There were 37 RA patients (35 females and 2 males) and 10 age and sex matched healthy controls with a mean age of 44.3 ± 2.91 years (9 females and 1 male). The mean IL-6 level for controls (7.71 ± 8.59 pg/ml) was significantly lower than the level in patients ($p = 0.044$). The demographic, clinical and laboratory features of the RA patients are presented in Table 1. Medications used are also presented in the table and all the patients were irregularly receiving declophenac sodium (50–100 mg) while only one was receiving full dose of sulphasalazine (2000 mg/day). Three of the patients were diabetic and hypertensive with two of them having hepatitis C virus (HCV) which was also present in another two patients. The rheumatoid factor was positive in 35 patients (94.6%) and anti-CCP was positive in 78.38%. None of the patients gave family history for any rheumatic disease.

There were 24 patients (64.86%) with GG IL-6 (−174 G/C) gene promoter polymorphism, 11 (29.73%) with GC and 2 (5.41%) with CC, while 9 (90%) of the control showed GG genotype with one individual with GC. The IL-6 was significantly higher in patients with CC (546 ± 343.65 pg/ml) compared to those with GG (12.54 ± 14.82 pg/ml) and GC (69.97 ± 113.23 pg/ml), ($p < 0.0001$) (Fig. 1).

Furthermore, there was a significant difference in the platelet count which was 268.54 ± 73.36 × 103/mm^3 for those with GG genotype, 335.36 ± 99.81 × 103/mm^3 for GC and being the highest (362.5 ± 36.06 × 103/mm^3) in those with CC ($p = 0.049$). Similarly, the more advanced the promoter polymorphism, the more the functional impairment of the patients as assessed by the HAQ-II score which was 0.6 ± 0.65 for those with GG genotype, 0.62 ± 0.19 with GC and 2 ± 1.41 for those with CC ($p = 0.029$). The modified Larsen score tended to be higher in those with CC genotype, however the difference was not significant. The RA patients with positive anti-CCP antibodies had a significantly higher modified Larsen score (41.32 ± 16.53) compared to those with negative test (21.25 ± 3.77) ($p = 0.001$); however the difference in DAS28 was insignificant ($p = 0.5$). There were no other significant differences in the characteristic features of the patients according to the promoter genes.

Those with associated HCV had a tendency to an elevated IL-6 level (275.35 ± 370.2 pg/ml) compared to those without (32.16 ± 69.98 pg/ml) ($p = 0.28$), higher DAS28 (5.63 ± 2.15 vs 4.22 ± 1.65, p = 0.28) and impaired functional status

Table 1 Demographic, clinical, laboratory and radiological features of RA patients.

Feature	RA patients No. (37)
	mean ± SD
Age (years)	44.49 ± 13.46
Disease duration (years)	8.91 ± 7.15
Morning stiffness (min.)	23.51 ± 38.94
No. of swollen joints	1.73 ± 3.16
No. of tender joints	5.89 ± 8.19
VAS (mm)	30.14 ± 32.61
ESR (mm/1st hr)	54.57 ± 24.4
Hemoglobin (g/dl)	11.23 ± 1.59
Platelets (x10^3/ml)	293.49 ± 86.35
IL-6 (pg/ml)	58.45 ± 147.09
Modified Larsen score	38.55 ± 16.94
DAS28	4.37 ± 1.73
HAQ-II	0.71 ± 0.73
Medications:	
Methotrexate (30/37) (mg/week)	14.59 ± 8.71 [17.5;0–25]
Prednisolone (15/37) (mg/day)	2.84 ± 3.69 [0;0–10]
Leflunomide (8/37) (mg/day)	4.05 ± 7.98 [0;0–20]
Chloroquine (16/37) (mg/day)	108.11 ± 125.56 [0;0–250]

VAS: visual analogue scale, ESR: erythrocyte sedimentation rate, IL-6: Interleukin 6, DAS28: Disease activities score for 28 joints, HAQ: health assessment questionnaire. Values between square brackets represent the median and range.

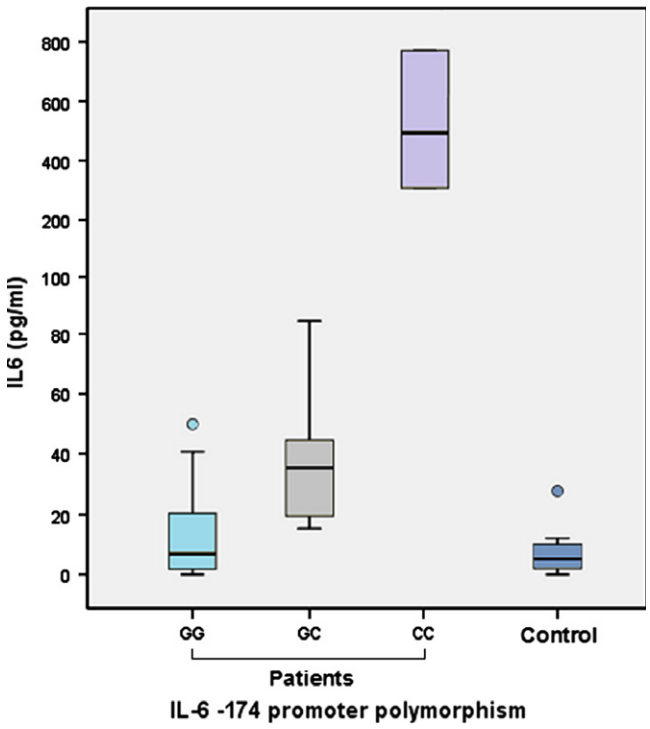

Figure 1 Box plot of the serum IL-6 level in the RA patients and control according to the IL-6 (−174 G/C) promoter polymorphism type.

assessed by HAQ-II (1.78 ± 0.93 vs 0.58 ± 0.6, $p = 0.077$), two of these patients had CC promoter polymorphism.

In the studied RA patients the IL-6 level significantly negatively correlated with the age at disease onset ($r = -0.34$, $p = 0.037$) while there was a tendency for this association between gene polymorphism and age at disease onset ($r = -0.32$, $p = 0.057$). The age at disease onset was older in those with GG genotype (38.29 ± 13.72 years), 32.59 ± 11.11 years in those with GC genotype and was younger in 2 patients with CC genotype (19.5 ± 3.54 years). In those with GG genotype,

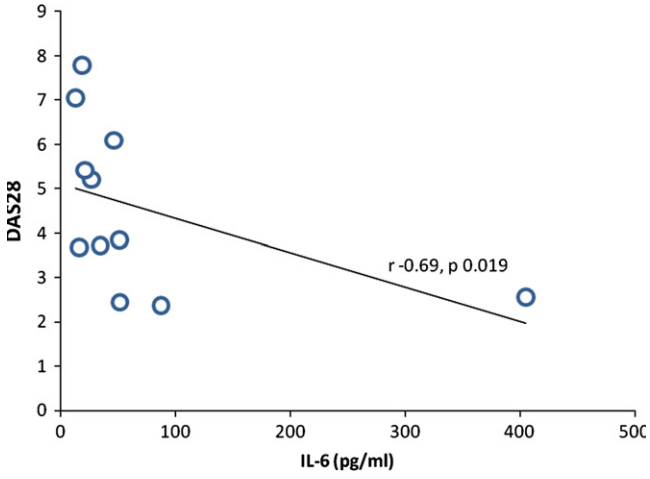

Figure 2 Correlation of the serum IL-6 level and the DAS28 in RA patients with GC −174 promoter polymorphism.

there was a significant correlation between IL-6 level and duration of the morning stiffness (MS) ($r = 0.44$, $p = 0.033$). While in those with GC genotype there was a significant negative correlation of the IL-6 level with the number of swollen joints ($r = -0.68$, $p = 0.22$), number of tender joints, VAS for pain ($r = -0.7$, $p = 0.017$), positive anti-CCP ($r = 0.34$, $p = 0.039$) and the DAS28 ($r = -0.69$, $p = 0.019$) (Fig. 2). Furthermore, the positivity of anti-CCP correlated with the modified Larsen score (r = 0.52, $p = 0.013$). No significant correlation was present between IL-6 level and disease duration, other clinical features (MS, number of swollen joints, number of tender joints, visual analogue score VAS), other laboratory investigations (erythrocyte sedimentation rate ESR, hemoglobin content Hb and platelet count), steroid dose and disease activity (DAS28). None of the studied clinical and laboratory parameters would predict the IL-6 promoter polymorphism.

4. Discussion

In the current study, the mean serum IL-6 level was significantly higher in RA patients than in control. In agreement, higher levels of IL-6 were present in serum, synovial tissue and synovial fluid from patients with RA compared to those with non-inflammatory arthritis [2,6]. Furthermore, there was a significant difference in the genotype and allele frequency at −174 of IL-6 between RA patients and healthy subjects [22]. On the other hand, another study found that the distribution of IL-6 genotypes in RA patients did not differ from that in control subjects [4]. Chronic joint inflammation in RA leads to the production of IL-6 and its receptor (IL-6R), which is expressed on effector cells that cause and prolong inflammation. Interleukin-6 is over-expressed in synovial tissue in RA patients, with raised concentrations in serum and synovial fluid [23].

In the present study on Egyptian RA patients, there were 64.86% with GG IL-6 (−174 G/C) gene promoter polymorphism, 29.73% with GC and 5.41% with CC, while 90% of the control showed GG genotype with one individual presenting with GC. In disagreement to the present study results, no significant differences in the IL-6 −174 allele or genotype frequencies between RA patients and controls were found. However, a role of IL-6 −174 gene polymorphism in the development of subclinical atherosclerosis in patients with RA was supported [13]. Moreover, another study found that the promoter polymorphism frequency of the IL-6 C allele was lower in RA patients than in controls [3]. There is a possible association between IL-6 promoter polymorphisms, at position −174, and outcome of RA. No difference was observed in the distribution of IL-6 promoter genotype or allele frequencies between RA patients and controls which appears to rule out an important role of IL-6 promoter polymorphisms in the susceptibility to RA in a Spanish population [8]. In a recent meta-analysis study, it was found that IL-6 −174 G/C polymorphism may confer susceptibility to RA in Europeans [24].

In the present study, the IL-6 was significantly higher in those with CC compared to those with GG and GC genotypes. Contrarily, allele −174 C is associated with a reduced IL-6 promoter strength of expression and IL-6 serum levels are lower in subjects with the CC genotype compared with GC or GG subjects [8]. Others stated that no definite association between gene polymorphisms and IL-6 serum levels was noticed [25].

In the present study, 4 patients had associated HCV and the level of IL-6 was higher compared to those without HCV. The difference was insignificant, however the number of patients included is small to withdraw a reliable conclusion. Two of these HCV associated patients had promoter polymorphism (IL6 −174 CC). The IL-6 level was found elevated in HCV patients and after clearing HCV infection, the level significantly decreased [26,27]. The high serum levels of IL-6 described in the course of RA have been linked to its enhanced activity. Furthermore, the high level in patients with HCV related arthritis indicates an increased synthesis and hyperactivity of this cytokine. The substantial similarity of the behavior of IL-6 in the RA and HCV-related arthritis patients suggests common mechanisms, in which the function of I L-6 is central [28]. It has been reported that IL-6 promoter polymorphisms influence the development of chronic HCV infection [29].

In the present study, the higher the promoter polymorphism, the more the functional impairment of the patients as assessed by the HAQ score and more the increased platelet count. In those with GG genotype, there was a significant correlation between IL-6 level and duration of the MS and in those with the GC genotype IL-6 showed a significant inverse correlation with the parameters of disease activity and the DAS28. The pro-inflammatory role of the IL6 system in established RA has been highlighted by the use of anti-sIL-6R antibodies [3]. However, a protective effect of IL6 on the risk of developing RA is suggested [3] which may further explain the inverse correlation in the present study. The consumption and involvement of IL-6 with its receptor in the active RA patients may further explain the inverse relation. On the other hand, in a previous study, IL-6 levels in RA patients correlated positively with disease activity [30]. In another study, in patients with a GG genotype, the active form of RA was more frequently diagnosed compared with CC and GC patients. Moreover, in carriers of two G alleles the parameters of DAS28, ESR, and number of swollen and tender joints were significantly increased [4]. Others found no significant relationship between IL-6 cytokine levels and DAS28 [6]. In RA patients, a similar apparent decline in DAS28 occurred with either abatacept or tocilizumab therapy except that the decreased disease activity was more rapid among tocilizumab-treated patients, directly caused by IL-6 inhibition [31]. Thrombopoietin acts as an acute phase protein but is not uniquely responsible for thrombocytosis of inflammatory disorders, while IL-6 positively correlated with the platelet count which makes it recognized as a credible candidate and as a cooperating factor [32]. Impairment of physical function and health-related quality of life, as well as fatigue (a major debilitating factor in RA), were all improved more with anti-IL6 tocilizumab than with placebo, reflecting substantial functional benefits for the patients [23].

In the present study, there was no correlation of the serum IL-6 level with the MS, however, in those with GG genotype there was a significant correlation. In RA patients with MS, there may be insufficient endogenous cortisol released during the night to counter elevated levels of Il-6 [33]. Some RA patients in remission still experience prolonged MS and thus it remains an important marker of active disease that should continue to be monitored [34]. Major improvement in MS was observed in RA patients treated by IL-6-receptor antagonist tocilizumab. Meanwhile, the disease activity did not differ between RF-positive and negative patients [35].

In the present study, there were no other significant differences in the characteristic features of the RA patients according to the promoter genes. Similarly, with regard to clinical and laboratory parameters, no differences were found according to the sex, duration, extraarticular manifestations, DAS28, baseline HAQ, RF and anti-CCP statuses according to the different IL-6 −174 genotypes [7,8].

In the studied RA patients, the IL-6 level significantly negatively correlated with the age at disease onset while there was a tendency for this association between gene polymorphism and age at disease onset. In harmony were the results that found an observed significant difference in the mean age at disease onset between IL-6 genotypes and considered that this points to the possible contribution to the pathogenesis by influencing the age at disease onset [8]. Interestingly, a significant difference in the mean age at disease onset was lower in patients at −174 CC than those at −174 GG and the age for heterozygous −174 CG was intermediate between means for homozygotes [8].

In the present study there was no significant difference in the Modified Larsen score among the RA patients according to the promoter genes however, the score tended to be higher in those with CC genotype. Contrarily, the IL6 −174G allele polymorphism is associated with more severe radiologic damage in RA [3]. Interleukin-6 gene (−174G/C) has been associated with bone erosive damage in RA patients especially those with CC genotype [36]. Of particular relevance to RA, IL-6 induces osteoclast differentiation, contributing to joint destruction, bone resorption and osteoporosis [23]. Targeting IL-6 seems an attractive therapeutic option in this disease, since it has a pivotal role in mediating inflammation, auto-antibody production, joint destruction and also systemic manifestations of RA. It is considered the most abundant cytokine in joints and serum of RA patients and its levels are correlated with the disease activity [23].

IL-6 (−174) promoter polymorphism may be a genetic risk factor determining the effectiveness of RA treatment with methotrexate and glucocorticoids as the incidence of remission after therapy was significantly lower in patients with GG genotype compared with GC and CC [37]. Targeting IL-6R with a humanized anti IL-6R monoclonal antibody (tocilizumab) effectively controls local and systemic inflammatory manifestations and blocks cartilage and bone destruction [5]. Blocking IL-6 can be a therapeutic option for patients with autoimmune diseases in which overproduction of IL-6 plays a pathological role and future clinical studies investigating the safety and efficacy will elucidate the clinical benefits [14]. Lack of response to rituximab was more prevalent in RA patients with the IL-6 −174 CC genotypes than in the GC/GG patients suggesting that IL-6 promoter genotyping may be useful to a better treatment plan with RTX in RA [7,25].

In conclusion, serum IL-6 levels and −174 G/C promoter polymorphism were higher in RA patients than in healthy controls. None of the studied parameters would predict the IL-6 level and promoter polymorphism. In those with IL-6 −174 GC promoter polymorphism, the increased disease activity was inversely related to the serum IL-6 level. The positive correlation of IL-6 with the duration of morning stiffness is of clinical significance. To confirm our results we propose that

larger scale, multicentre studies with longer evaluation periods are needed.

Conflicts of interest

None.

References

[1] Perricone C, Ceccarelli F, Valesini G. An overview on the genetic of rheumatoid arthritis: a never-ending story. Autoimmun Rev 2011;10(10):599–608.

[2] Nile CJ, Read RC, Akil M, Duff GW, Wilson AG. Methylation status of a single CpG site in the IL6 promoter is related to IL6 messenger RNA levels and rheumatoid arthritis. Arthritis Rheum 2008;58(9):2686–93.

[3] Marinou I, Healy J, Mewar D, Moore DJ, Dickson MC, Binks MH, et al. Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. Arthritis Rheum 2007;56:2549–56.

[4] Pawlik A, Wrzesniewska J, Florczak M, Gawronska-Szklarz B, Herczynska M. IL-6 promoter polymorphism in patients with rheumatoid arthritis. Scand J Rheumatol 2005;34(2):109–13.

[5] Fonseca JE, Santos MJ, Canhão H, Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. Autoimmun Rev 2009;8(7):538–42.

[6] Chung SJ, Kwon YJ, Park MC, Park YB, Lee SK. The correlation between increased serum concentrations of interleukin-6 family cytokines and disease activity in rheumatoid arthritis patients. Yonsei Med J 2011;52(1):113–20.

[7] Fabris M, Quartuccio L, Lombardi S, Benucci M, Manfredi M, Saracco M, et al. Study on the possible role of the −174G > C IL-6 promoter polymorphism in predicting response to rituximab in rheumatoid arthritis. Reumatismo 2010;62(4):253–8.

[8] Pascual M, Nieto A, Matarán L, Balsa A, Pascual-Salcedo D, Martín J. IL-6 promoter polymorphisms in rheumatoid arthritis. Genes Immun 2000;1(5):338–40.

[9] Marinou I, Walters K, Winfield J, Bax DE, Wilson AG. A gain of function polymorphism in the interleukin 6 receptor influences RA susceptibility. Ann Rheum Dis 2010;69(6):1191–4.

[10] Jones KG, Brull DJ, Brown LC, Sian M, Greenhalgh RM, Humphries SE, et al. Interleukin-6, (IL-6) and the prognosis of abdominal aortic aneurysms. Circulation 2001;103:2260–5.

[11] Humphries SE, Luong LA, Ogg MS, Hawe E, Miller GJ. The interleukin-6 −174 G/C promoter polymorphism is associated with risk of coronary heart disease and systolic blood pressure in healthy men. Eur Heart J 2001;22(24):2243–52.

[12] Mosaad YM, Fathy H, Fawzy Z, El-Saied MA. Tumor necrosis factor-α −308 G > A and interleukin-6 −174 G > C promoter polymorphisms and pemphigus. Hum Immunol 2012;73(5): 560–5.

[13] Palomino-Morales R, Gonzalez-Juanatey C, Vazquez-Rodriguez TR, Miranda-Filloy JA, Llorca J, Martin J, et al. Interleukin-6 gene −174 promoter polymorphism is associated with endothelial dysfunction but not with disease susceptibility in patients with rheumatoid arthritis. Clin Exp Rheumatol 2009;27(6): 964–70.

[14] Murakami M, Nishimoto N. The value of blocking IL-6 outside of rheumatoid arthritis: current perspective. Curr Opin Rheumatol 2011;23(3):273–7.

[15] Assier E, Boissier MC, Dayer JM. Interleukin-6: from identification of the cytokine to development of targeted treatments. Joint Bone Spine 2010;77(6):532–6.

[16] Nishimoto N, Yoshizaki K, Miyasaka N, Yamamoto K, Kawai S, Takeuchi T, et al. Treatment of rheumatoid arthritis with humanized anti–interleukin-6 receptor antibody: a multicenter, double blind, placebo-controlled trial. Arthritis Rheum 2004;50:1761–9.

[17] Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham 3rd CO, et al. 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Arthritis Rheum 2010;62(9):2569–81.

[18] Prevoo MLL, Hof van't MA, Kuper HH, Leeuwen van MA, Putte van de LBA, Riel van PLCM. Modified disease activity scores that include twenty-eight-joint counts: development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 1995;38:44–8.

[19] Wolfe F, Michaud K, Pincus T. Development and validation of the Health Assessment Questionnaire II: a revised version of the Health Assessment Questionnaire. Arthritis Rheum 2004;50:3296–305.

[20] Larsen A. How to apply Larsen score in evaluating radiographs of rheumatoid arthritis in long term studies. J Rheumatol 1995;22:1974–5.

[21] Pola R, Flex A, Gaetani E, Pola P, Bemabei R. The −174 G/C polymorphism of the interleukin-6 gene promoter and essential hypertension in an elderly Italian population. J Hum Hypertens 2002;16(9):637–40.

[22] Huang XZ, Zhuang JH, Ren YG, Zhou LJ, Zhou Q. Association of interleukin-6 and interleukin-18 gene polymorphism with rheumatoid arthritis in Guangdong Han population. Nan Fang Yi Ke Da Xue Xue Bao 2007;27(11):1661–4.

[23] Brandão FM, Coelho P, Pinto P, Combe B. Tocilizumab – a new step in rheumatoid arthritis treatment. Acta Reumatol Port 2010;35(3):302–12.

[24] Lee YH, Bae SC, Choi SJ, Ji JD, Song GG. The association between interleukin-6 polymorphisms and rheumatoid arthritis: a meta-analysis. Inflamm Res 2012;61(7):665–71.

[25] Fabris M, Quartuccio L, Lombardi S, Saracco M, Atzeni F, Carletto A, et al. The CC homozygosis of the −174G > C IL-6 polymorphism predicts a lower efficacy of rituximab therapy in rheumatoid arthritis. Autoimmun Rev 2012;11(5):315–20.

[26] Afzal N, Abbas S, Ahmed A, Arif M, Javeed K. Effect of hepatitis C virus on C-reactive protein and interleukin-6 in hemodialysis patients. Iran J Kidney Dis 2011;5(3):182–6.

[27] Guzmán-Fulgencio M, Jiménez JL, Berenguer J, Fernández-Rodríguez A, López JC, Cosín J, et al. Plasma IL-6 and IL-9 predict the failure of interferon-α plus ribavirin therapy in HIV/HCV-coinfected patients. J Antimicrob Chemother 2012;67(5):1238–45.

[28] Riccio A, Postiglione L, Sabatini P, Linvelli M, Soriente I, Sangiolo MG, et al. Similar serum levels of IL-6 and its soluble receptors in patients with HCV-related arthritis and rheumatoid arthritis: a pilot study. Int J Immunopathol Pharmacol 2012;25(1):281–5.

[29] Cussigh A, Falleti E, Fabris C, Bitetto D, Cmet S, Fontanini E, et al. Interleukin 6 promoter polymorphisms influence the outcome of chronic hepatitis C. Immunogenetics 2011;63(1):33–41.

[30] Van Leeuwen MA, Westra J, Limburg PC, van Riel PL, van Rijswijk MH. Clinical significance of interleukin-6 measurement in early rheumatoid arthritis: relation with laboratory and clinical variables and radiological progression in a three year prospective study. Ann Rheum Dis 1995;54:674–7.

[31] Leffers HC, Ostergaard M, Glintborg B, Krogh NS, Foged H, Tarp U, et al. Efficacy of abatacept and tocilizumab in patients with rheumatoid arthritis treated in clinical practice. results from the nationwide Danish DANBIO registry. Ann Rheum Dis 2011;70(7):1216–22.

[32] Ceresa IF, Noris P, Ambaglio C, Pecci A, Balduini CL. Thrombopoietin is not uniquely responsible for thrombocytosis in inflammatory disorders. Platelets 2007;18(8):579–82.

[33] Buttgereit F. How should impaired morning function in rheumatoid arthritis be treated? Scand J Rheumatol Suppl 2011;125: 28–39.

[34] Sierakowski S, Cutolo M. Morning symptoms in rheumatoid arthritis: a defining characteristic and marker of active disease. Scand J Rheumatol Suppl 2011;125:1–5.

[35] Burmester GR, Feist E, Kellner H, Braun J, Iking-Konert C, Rubbert-Roth A. Effectiveness and safety of the interleukin 6-receptor antagonist tocilizumab after 4 and 24 weeks in patients with active rheumatoid arthritis: the first phase IIIb real-life study (TAMARA). Ann Rheum Dis 2011;70(5):755–9.

[36] Ceccarelli F, Perricone C, Fabris M, Alessandri C, Iagnocco A, Fabro C, et al. Transforming growth factor-beta 869C/T and interleukin-6 −174G/C polymorphisms relate to the severity and progression of bone erosive damage detected by ultrasound in rheumatoid arthritis. Arthritis Res Ther 2011;13(4):R111.

[37] Pawlik A, Czerny B, Dabrowska-Zamojcin E, Górnik W, Poziomkowska I, Gawrońska-Szklarz B, et al. The influence of IL-6 polymorphism on efficacy of treatment of rheumatoid arthritis patients with methotrexate and prednisone. Pol Arch Med Wewn 2005;114(3):843–7.