The Egyptian Journal of Medical Human Genetics 19 (2018) 235–240

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Contents lists available at ScienceDirect

The Egyptian Journal of Medical Human Genetics

journal homepage: www.sciencedirect.com

Original article

Association of interleukin-6 and its -174G/C promoter polymorphism with clinical and laboratory characteristics of non hepatitis C virus rheumatoid arthritis patients

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ARTICLE INFO

Article history:
Received 30 November 2017
Accepted 25 December 2017
Available online 5 January 2018

Keywords:
IL-6
HCV
(-174G/C) Promoter polymorphism
Rheumatoid arthritis

ABSTRACT

Background: Interleukin-6 is a cytokine protein, which causes inflammation, maintains immune homeostasis and shows a role in rheumatoid arthritis pathogenesis. IL-6-174G/C promoter polymorphism may have a role in susceptibility to RA.
Aim of the work: To evaluate the clinical significance of serum levels of IL-6 and its -174G/C promoter polymorphism in RA patients in comparison with the controls.
Patients and methods: This study enrolled 25 non hepatitis C virus RA patients versus 25 age and gender matched controls. Demographic, clinical and laboratory data were prospectively evaluated. Serum IL-6 level and promoter (-174G/C) genotype were determined.
Results: Serum IL-6 levels was significantly higher in RA patients compared to control subjects (p = .001), especially those with CC promoter polymorphism. There was a significant correlation between IL and 6 level and duration of morning stiffness, disease activity, hemoglobin concentration and ESR level. 15/25 patients had (-174G/G) gene promoter polymorphism, 8/25 were GC and 2/25 were CC. All controls were GG. There was significant association between gene polymorphism and age at disease onset (p = .0172), which was older in those with GG genotype (38.5 ± 10.25 years) than those with CC (33.5 ± 0.7 1 years) and younger in GC genotypes (27.9 ± 7.9 years). None of the other clinical, laboratory or radiological parameters would predict the IL-6 promoter polymorphism.
Conclusion: Serum IL-6 levels and -174G/C promoter polymorphism were higher in RA patients than in healthy controls. The positive correlation of IL-6 level with the DAS28 and duration of morning stiffness may confirm its' increased involvement in the pathogenesis of RA and may point to the need for considering of anti-IL-6 agents in their management plan. The negative correlation of IL-6 level with the hemoglobin level may confirm IL-6 play a significant role in anemia of RA.

1. Introduction

Autoimmune diseases are major causes of morbidity and mortality in the industrialized world. Autoimmunity develops after breaking self-tolerance of the immune system [1].

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease affecting joints mainly. Although it is primarily considered a disease of the joints, abnormal systemic immune responses are evident and can cause a variety of extra articular manifestations [2]. RA has been known as a multifactorial disease sustained by environmental and genetic factors. These factors seem to be necessary although not sufficient in disease development. However, these factors can be responsible for different clinical pictures and response to therapy. Several genes have been incriminated so far in the pathogenesis of RA. Elevated levels of pro-inflammatory cytokines are key features in patients with RA [3].

Abnormalities in cytokines, their receptors, and their signaling pathways are involved in a wide variety of diseases. Solid evidences have implicated IL-6 in the pathogenesis of RA [4]. IL-6

Abbreviations: ACPA, anti-citrullinated protein antibodies; ACR/EULAR, American College of Rheumatology/European League Against Rheumatism; CBC, complete blood count; DAS28, Disease activity score 28; ESR, erythrocyte sedimentation rate; HB, hemoglobin level; HCV, Hepatitis C Virus; IL-1, Interleukin-1; PCR-RFLP, Polymerase chain reaction-restriction fragment length polymorphism; RA, Rheumatoid arthritis; RF, rheumatoid factor; SC, subcutaneous nodules; SNPs, single nucleotide polymorphisms; TNF-α, Tumor necrosis factor-alpha.

Peer review under responsibility of Ain Shams University.

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https://doi.org/10.1016/j.ejmhg.2017.12.004
1110-8630/

has a substantial role in synovitis, bone erosions and in the systemic features of inflammation [5]. Its serum concentration is significantly elevated in RA patients and decreases with medical treatment proposing its role in the pathogenesis of RA [6]. Furthermore, its-174 promoter polymorphism is associated with disease susceptibility and activity and also constitutes a genetic risk factor [7,8]. Single nucleotide polymorphisms (SNPs) in IL-6 genes -174G/C have been associated with RA susceptibility and radiographic severity of bone-erosive damage [6]. The "anti-cytokine medicine" is a rapidly growing field that may dramatically affect disease management. Tocilizumab, a recombinant humanized monoclonal IgG1 antihuman interleukin 6-receptor antibody is now indicated for the treatment of adults with RA who have failed to respond to at least one synthetic disease-modifying anti-rheumatic drug or TNFα antagonist [9]. The discovery of new genes associated with the disease may play a crucial role in understanding the development, progression and outcome of RA [3].

Hepatitis C Virus (HCV) still affects a substantial proportion of the Egyptian population. It is estimated that in the 15- to 59-year age groups, the prevalence of HCV antibody was 10.0% and that HCV RNA was 7.0%. In children, 1–14 years old, the prevalence of HCV antibody and HCV RNA were 0.4% and 0.2% respectively. Approximately, 3.7 million persons have chronic HCV infection in the age group 15–59 in 2015[10]. HCV is a hepatotropic lymphotropic virus. Lymphotropism and chronic stimulation of the immune system by several viral proteins may be responsible for non-organ specific autoantibody production as rheumatoid factor (RF) and cryoglobulins [11]. Elevated levels of interleukin (IL-6) levels are observed in rheumatoid and HCV-related arthritis. However, this increase is not related to HCV viremia [12]. Anti-cyclic citrullinated peptide (ACCP) positivity is considered specific for a differential diagnosis of arthritis in patients infected with HCV and is more significant for rheumatoid arthritis than the other causes [12]. Rheumatologic extrahepatic manifestations are observed in many HCV-infected patients. These include arthralgia (23%), paresthesia (17%), myalgia (15%), pruritus (15%), and sicca syndrome (11%) [13].

2. Aim of the work

To evaluate the clinical significance of serum levels of IL-6 and its -174G/C promoter polymorphism in non HCV RA patients in comparison with the controls.

3. Subjects & methods

3.1. Study population

Twenty-five RA patients diagnosed according to the 2010 ACR/EULAR RA classification criteria [14] were consecutively recruited from the Rheumatology outpatient clinic and department of Cairo University Hospitals versus 25 age and gender matched healthy controls. 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/or ACCP and plain X-rays for the affected joints were assessed. Disease activity score (DAS-28) was calculated [15]. Informed consent was taken from each patient. The work has been carried out in accordance with The Code of Ethics of The World Medical Association of Helsinki for experiments in humans.

3.1.1. Patient exclusion criteria

Patient with HCV were excluded as HCV can cause arthritis which may be misdiagnosed as RA. Also hepatitis may increase the level of IL-6. All patients were negative for HCV antibodies.

3.2. Methods

3.2.1. Determination of serum IL-6 level

Serum IL-6 was assayed using Human IL-6 ELISA kit (BOSTER BIOLOGICAL TECHNOLOGY Co., Ltd. 3942 B Valley Ave, Pleasanton, California America, 94566).

3.2.2. Determination of IL-6 gene (-174G/C) promoter polymorphism

Promoter region polymorphism of IL-6 gene (-174G/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 using (ZYMO RESEARCH CORP) whole Blood Genomic DNA Extraction Kit.

Enzymatic amplification was performed by PCR using Master Taq polymerase enzyme (Bio-25044mytaq red mix Meridian Bioscience Asia Pte Ltd, SINGAPORE) and Biometra T personal thermal cycler. Amplification of the promoter region (-174G/C) of the IL-6 gene was done as proposed by Pola et al. [16] using 2 primers 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 (50 μl) contained 25 μl MyTaq™ Red Mix2x (MyTaq Red Mix, 2x is a pre-mixed solution containing Thermus aquiticus (Taq) DNA polymerase, PCR buffer, dNTP, gel loading dyes and fluorescence dye), 1 μl of each primer (25 pmol), 5 μl of genomic DNA and 18 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 63 °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 NLaIII restriction enzyme (Time-Saver™ Qualified (New England biolabsinc, USA). The digested products were detected in 2% agarose gel containing ethidium bromide by performing electrophoresis on the gel electrophoresis apparatus and were visualized by UV transillumination. 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 identified a GC heterozygote.

3.3. Statistical analysis

Data were coded and entered using the statistical package SPSS version 23. Data were summarized using mean, standard deviation, median, minimum and maximum in quantitative data and using frequency (count) and relative frequency (percentage) for categorical data. Comparisons between quantitative variables were done using the non-parametric Kruskal-Wallis and Mann-Whitney tests. For comparing categorical data, Chi square test was performed. Exact test was used instead when the expected frequency is less than 5. Correlations between quantitative variables were done using Spearman correlation coefficient). *P-values* less than .05 were considered as statistically significant.

4. Results

Fifty subjects were enrolled in this study; 25 RA (23 females and 2 males) and 25 age and sex matched healthy controls with a mean age of 48.04 ± 14.54 years (23 females and 2 male). The clinical features, laboratory results, radiological findings and medications used at the time of the study are shown in Table 1 and Table 2.

The mean IL-6 level was significantly higher in the patients group (50.59 ± 43.33 pg/ml) compared with the control group (13.32 ± 7.67 pg/ml) ($p < .001$).

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

	Mean	Standard deviation	Median	Minimum	Maximum
Age (years)	43.96	12.14	42.00	25.00	66.00
Onset (years)	8.96	8.64	6.00	2.00	31.00
Platelets ($\times 10^3$/ml)	260.72	81.64	237.00	163.00	420.00
TLC* ($\times 10^3$/ml)	7.24	2.32	7.50	3.40	12.40
Hemoglobin (g/dl)	11.45	1.59	11.70	7.90	15.20
ESR** (mm/1st hr)	48.00	26.47	46.00	12.00	105.00
Swollen joint	4.24	6.02	1.00	.00	22.00
Tender joint	6.28	8.22	3.00	.00	27.00
DAS28***	4.11	1.49	3.66	1.98	7.16
Morning stiffness (min.)	32.40	68.36	.00	.00	240.00

* TLC: total leukocyte count.
** ESR: erythrocyte sedimentation rate.
*** DAS28: disease activities score for 28 joints.

Table 2
Clinical, laboratory and radiological features of RA patients.

		Count	%
Rheumatoid factor	Positive	20	80.0%
	Negative	5	20.0%
DAS28*	Remission	4	16.0%
	Low	3	12.0%
	Moderate	13	52.0%
	High	5	20.0%
X-RAY erosion	Yes	13	52.0%
	No	12	48.0%
Subcutaneous nodule	Yes	4	16.0%
	No	21	84.0%
Methotrexate	Yes	23	92.0%
	No	2	8.0%
Leflunomide	Yes	5	20.0%
	No	20	80.0%
Chloroquine	Yes	10	40.0%
	No	15	60.0%
Prednisolone	Yes	13	52.0%
	No	12	48.0%

* DAS28: disease activities score for 28 joints.

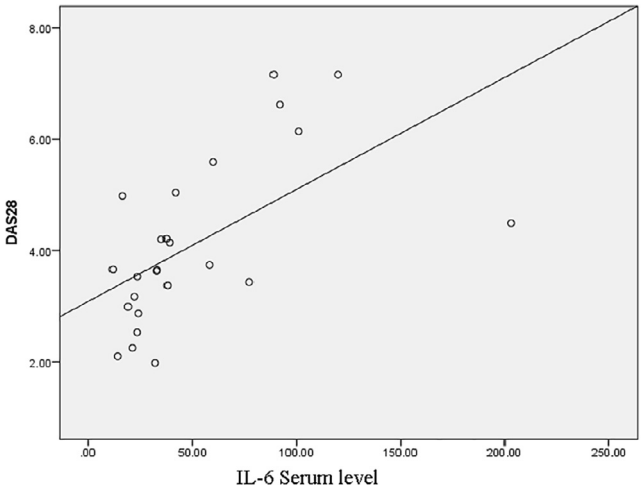

Fig. 1. Correlation between patients' IL-6 serum level and DAS28.

There was a high statistically significant difference between the patients group & the control group regarding IL-6 (-174G/C) promoter polymorphism types (p = .001). Fifteen patients (60%) were GG IL-6 (-174G/C) genotype, 8 (32%) were GC and 2 (81%) were CC genotype. However, all the 25 controls subjects were GG genotype.

Serum IL-6 was higher in patients with CC genotype (76 ± 62. 23 pg/ml) compared to those with GG genotype (48.56 ± 48.01 p g/ml) and GC genotype (48.05 ± 33.24 pg/ml), yet no statistically significant difference was observed (p = *.716*).

There was a statistically significant difference between gene polymorphism and age at disease onset (p = .0172). Older ages of disease onset were those harboring GG genotype (38.5 ± 10.25 ye ars), followed by CC genotype (33.5 ± 0.71 years) and finally those with GC genotype (27.9 ± 7.9) years.

However, there was no correlation between IL and 6 serum level with the age at disease onset (r = 0.1678, p = .423).

Bone erosions revealed by X-ray were detected in all patients with CC genotype 2 (100%) compared to 7 patients (46%) with GG allele and 4 patients (50%) with GC allele. However, of no significance (p = .580). While no significant correlation was found between IL and 6 level with bone erosion.

There was a positive correlation between IL and 6 level with DAS28 (r = 0.692, p < .001) (Fig. 1), number of swollen joints (r = 0.68, p = .003) and number of tender joints (r = 0.677, p < .001). However the difference in DAS28, number of swollen joints and number of tender joints among the different alleles were statistically insignificant (p = .943), (p = .979) and (p = .826) respectively.

Regarding the correlation between IL and 6 (174G/C) promoter polymorphism with the duration of morning stiffness, although not statistically significant, yet those with GG genotype had a higher duration of morning stiffness (42 min) compared to the GC genotype (19 min) and CC genotype (7 min) (p = .766). However there was a significant correlation between IL and 6 level and duration of morning stiffness (r = 0.607, p = .001) (Fig. 2) Also when analyzing IL-6 (174G/C) promoter polymorphism with appearance of subcutaneous nodules (SC) between the different alleles, although not statistically significant, yet 50% patients with CC allele were shown to have subcutaneous nodules in comparison to 6.7% patients with GG allele and 25% patients with GC allele (p = .167). Moreover there was no significant correlation between serum IL-6 level and the appearance of subcutaneous nodules.

As for various laboratory variables in RA patients, there was a positive correlation between IL and 6 level with ESR (r = 0.402, p = *.047*). While a negative correlation was found with hemoglobin level (HB) (r = −0.582, p = *.002*). However, no correlation was found between IL and 6 (174G/C) promoter polymorphism with either ESR or HB.

No significant association was found between IL and 6 or its (-174G/C) promoter polymorphism with platelet count, total leukocyte count, RF or treatment with methotrexate, Chloroquine and/or Prednisolone. None of the studied clinical and laboratory parameters would predict the IL-6 promoter polymorphism.

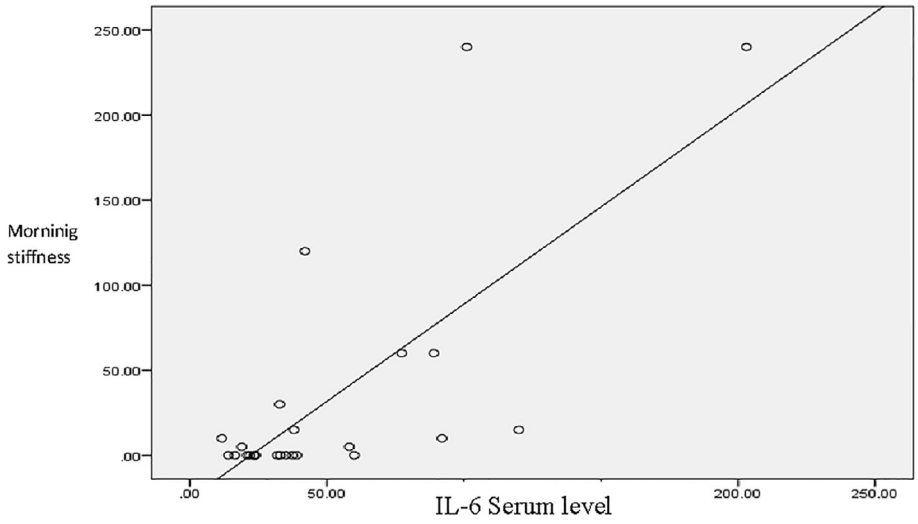

Fig. 2. Correlation between patients' IL-6 serum level and duration of morning stiffness.

5. Discussion

In the current study, the mean serum IL-6 level which was significantly higher in 25 RA patients (50.59 ± 43.44 pg/ml) compared to 25 sex and age matched control subjects (13.32 ± 7.67 pg/ml) (p = .001). In agreement with our study, Gaber et al. (2013) observed that the mean IL-6 level for 37 Egyptian RA patients (58. 45 ± 147.09 pg/ml) was significantly higher than its level in the control group (10 subjects) (7.71 ± 8.59 pg/ml) (p = .044) [17]. This is in concordance with Chung et al. (2011) who found that in 40 Korean patients IL-6 serum level was significantly higher than their 40 matched controls [6]. Similarly, 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 [4].

Malaguarnera et al. (1996) showed that serum IL-6 levels were more elevated in patients with liver disease than in healthy subjects, and that IL-6 levels increased as the histological activity of the liver disease progressed [18].

HCV-related arthritis can present with joint involvement symptoms similar to RA. Thus, clinically, it may be almost impossible to distinguish HCV-related arthritis from RA [19]. Rheumatoid factor (RF) is antibody produced by the immune system that can attack healthy tissue in the body. RF positivity is approximately 70–80% in patients with RA, and in HCV-related arthritis, this positivity ranges between 54% and 82% [20]. Moreover, RF is nonspecific and may be present in several diseases. On the other hand, anti-citrullinated protein antibodies (ACPA) are autoantibodies (antibodies to an individual's own proteins) that are directed against peptides and proteins that are citrullinated. ACPA are more specific for RA (96–98%) and are present in approximately 75–80% patients with RA. However, it has been shown that ACPA are present in 4.5% to 7% of patients with HCV-related arthritis [20]. For that reason HCV rheumatoid arthritis patients were excluded from our study.

In the present study there was a highly statistically significant difference between patient group & control group regarding IL-6 (-174G/C) promoter polymorphism types. For the patient group 60% were shown to have GG IL-6 (-174G/C) genotype, 32% with GC genotype and 8% with CC genotype while 100% of the control showed GG genotype (p = .001). In agreement with our results another study on 37 Egyptian RA patients reported that 64.86% of the patients were shown to have GG allele, 29.73% with GC and 5.41% with CC, while 90% of the control showed GG genotype with one individual presenting with GC [17]. Similarly, another study conducted on Chinese people found significant difference in the genotypes resulting from IL to 6 (-174G/C) gene promoter polymorphism between RA patients and healthy subjects [21]. On the other hand, another study included 98 patients with RA in Poland reported that among the studied group 53 (54.1%) were heterozygous GC, 26 (26.5%) were homozygous GG and homozygous CC genotype was observed in 19 (19.4%) cases. The same study found that among the studied group of control subjects (105) the genotypes GC, GG and CC were found in 58 (55.2%), 25 (23.8%) and 22 (21.0%) subjects, respectively. As shown, the study concluded that the distribution of IL-6 genotypes in RA patients did not differ significantly from that in the control group [7]. Similarly, in Spain Palomino-Morales et al. (2009) conducted their study on 311 RA patients and 226 matched controls. They found no significant differences in the IL-6 -174 allele and its genotype frequencies between RA patients and the control group [22]. Details they showed that GC allele ranked first followed by GG and finally CC in the patient group, while in the control group GG showed the highest frequency followed by GC and CC. However, Marinou et al. (2007) in United Kingdom reported that the frequency of the CC IL-6 (-174G/C) gene promoter polymorphism was lower in RA patients than in the controls [7]. A *meta*-analysis study found that IL-6-174G/C polymorphism may confer susceptibility to RA in Europeans [23]. While another meta-analysis study explained this divergence by the different genetic backgrounds. For instance, the -174C allele and its related genotypes were exceedingly lower in Asians and Eastern Chinese than in Europeans and other regions, respectively. Actually, it is now believed that one gene variant may play a different role in RA risk across different populations and regions [24].

In the present study, there was no statistically significant correlation between IL and 6 serum levels and IL-6 (-174G/C) promoter polymorphism types (p = .716) although the IL-6 serum level was higher in those with CC genotype compared to those with GG and GC genotype. Nevertheless, Gaber et al. (2013) found significant correlation between IL and 6 serum level and IL-6 (-174G/C) promoter polymorphism types which was significantly higher in CC genotype (546 ± 343.65 pg/ml) compared to those with GC genotype (69.97 ± 113.23 pg/ml), (p < .0001) and GG genotype (12.54 ± 14.82 pg/ml). Contrarily in Spanish population, -174 CC genotype was associated with a reduced IL-6 promoter strength of expression and IL-6 serum levels were lower in subjects with the CC genotype compared with GC or GG subjects [25]. Others stated that no definite association between gene polymorphisms and IL-6 serum level was noticed [26].

Regarding the correlations between IL and 6 serum level with the clinical manifestations and laboratory findings of RA patients: we found that there was a significant correlation between IL and 6 level and duration of morning stiffness, number of swollen joints, number of tender joints, DAS 28, ESR and hemoglobin level p values .001, .003, .001, .001, .047 and .002 respectively. In agreement with the present study a study in Netherlands, found that among 51 RA patients there was a significant correlation between IL and 6 level with ESR ($r = 0.744$, $p < .001$), hemoglobin level ($r = -0.31$ 1, $p < .05$) and duration of morning stiffness ($p < .05$). Moreover, IL-6 level in RA patients is correlated positively with disease activity ($p < .05$) [27]. Previous studies have also found a significant correlation between serum IL-6 activity and the serum levels of various acute phase reactants. The pro-inflammatory role of the IL6 system in established RA has been highlighted by the use of anti-sIL-6R antibodies; however, a protective effect of IL-6 on the risk of developing RA has been suggested [7]. On the other hand Gaber et al. (2013) found no significant relationship between IL and 6 levels and DAS28 ($p = .5$) [17]. Another study by Chung et al. (2011) found a significantly positive correlation of serum concentrations of IL-6 with CRP levels ($r = 0.45$, $p = .007$), but not with DAS28 ($r = 0.28$, $p = .102$); However, After eight weeks of medical treatment in patients with high disease activity, a decrease in DAS28 was associated with a significant decrease in the serum concentrations of IL-6 and IL-11 [6].

In the present study, there was positive correlation of the serum IL-6 level with the duration of morning stiffness; those with GG genotype had a higher duration (42 min) than GC genotype (19 min) and CC genotype (7 min). Another study on patients with GG genotype found a significant correlation between IL and 6 level and duration of morning stiffness ($r = 0.44$, $p = .033$) [17]. This condition may be attributed to the limited ability of endogenous cortisol released during the night to counter the high levels of Il-6 [28]. Some RA patients in remission still experience prolonged duration of morning stiffness and thus it remains an important marker of active disease that should continue to be monitored [29]. Major improvement in duration of morning stiffness was observed in RA patients treated by IL-6-receptor antagonist tocilizumab [29].

In the present study, there was no significant correlation of the serum IL-6 level with platelet count ($r = 0.172$, $p = .411$). Also Gaber et al. (2013) found no significant correlation of the serum IL-6 level with platelet count [17]. Contrarily, a study by Van Leeuwen et al. (1995) observed a significant correlation between IL and 6 level and platelet count ($r = 0.744$, $p < .001$) [27]. Although thrombopoietin acts as an acute phase protein but is not exclusively responsible for thrombocytosis of inflammatory disorders, the positive correlation of IL-6 serum level with the platelet count makes it a recognized reliable candidate and as a cooperating factor [30].

As for rheumatoid factor in the present study, we found no significant differences between Il and 6 serum level ($p = .272$) or promoter genes ($p = 1$) with RF-positive and negative patients Moreover, the disease activity did not differ between RF-positive and negative patients. In agreement with Burmester et al. (2011) in their study on Korean patients [31].

In our study, we found IL-6 level non significantly correlated with the age at disease onset ($r = 0.168$, $p = .423$). However, we found association between gene polymorphism and age at disease onset ($p = .0172$). The age at disease onset was older in those with GG genotype (38.5 ± 10.25 years), in comparison to those with CC genotype (33.5 ± 0.71 years) and those with GC genotype (27.9 ± 7.9 years). However, Gaber et al. (2013) found that IL-6 level was inversely correlated with the age at disease onset ($r = -0.34$, $p = .037$) and there was tendency for association between gene polymorphism and age at disease onset ($r = -0.32$, $p = .057$) [17].

In the present study, when comparing IL-6 (-174G/C) promoter polymorphism types with patients' radiological findings and treatment data; no statistically significant difference regarding joint or bone erosion by X-ray was found; however, all patients with CC genotype had bone erosion which was present in only 50% in the other genotypes. In agreement with the results of Gaber et al. (2013) who utilized the modified Larsen score which detect bone erosion found that it tended to be higher in those with CC genotype, but the difference was not significant [17]. Supporting our results Ceccarelli et al. (2011) found that bone erosive damage in 77 Italian RA patients was significantly correlated with those with CC genotype ($p = .007$) [32]. Of particular relevance to RA, IL-6 induces osteoclast differentiation, contributing to joint destruction, bone resorption and osteoporosis [33].

In our study there was no statistical association between neither IL-6 serum level nor IL-6 -174G/C promoter polymorphism and treatment with methotrexate ($p = .06$, $p = 1$), Chloroquine ($p = .285$, $p = 1$) and/or Prednisolone ($p = .936$, $p = .494$). Similarly, Gaber et al. (2013) found no statistically significant association between IL and 6 serum level or its -174G/C promoter polymorphism and treatment with methotrexate, Chloroquine and/or Prednisolone [17]. IL-6 is considered the most abundant cytokine in the joints and serum of RA patients and its level correlates with disease activity [33]. Pawlik et al. (2005) suggested that 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 genotypes [7]. Targeting IL-6R with a humanized anti IL-6R monoclonal antibody (tocilizumab) successfully controls local and systemic inflammatory manifestations and prevents cartilage and bone destruction [5]. Impairment of physical function and health-related quality of life, as well as fatigue, were all improved more with tocilizumab than with placebo, reflecting substantial functional benefits for the patients [33].

In the present study, when comparing IL-6 (-174G/C) promoter polymorphism types with patients' clinical and laboratory data; there was no statistically significant difference regarding the onset of disease ($p = .237$), number of swollen joint ($p = .979$), number of tender joints ($p = .826$), DAS28 ($p = .943$), subcutaneous nodule ($p = .167$), platelet count ($p = .930$), TLC ($p = .703$), hemoglobin level ($p = .3$) and ESR ($p = .974$). Similarly, many other authors reported no difference in clinical and laboratory parameters, regarding the duration, extra-articular manifestations, DAS28, and laboratory parameters (ESR, HB level and platelet count) in the different IL-6 -174 genotypes[17,25,26]. However, Pawlik et al. (2005) found that, in patients with a GG genotype, the active form of RA was more frequently diagnosed compared with CC and GC patients. Moreover, in GG genotype carriers the parameters of DAS28, ESR, and number of swollen and tender joints were significantly increased [7].

6. Conclusions

Serum IL-6 levels and its-174G/C promoter polymorphism were higher in Egyptian RA patients than in Egyptian healthy controls. None of the studied parameters would predict the promoter polymorphism except age at disease onset. The data from the present study indicate that a rare variant (C allele) in IL-6-174G/C promoter polymorphism may play a significant role in genetic susceptibility for RA in the Egyptian population. The positive correlation of IL-6 with the DAS28 and duration of morning stiffness may confirm its increased involvement in the pathogenesis of RA and may point to the need for considering of anti-IL-6 agents in the management plan. Further studies with larger sample size are required to delineate the differences in genetic susceptibility to RA in various ancestral groups.

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