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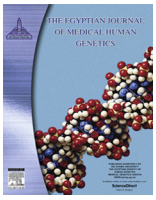

Original article

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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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 ± 0.71 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.

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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. Subjects & methods

2.1. 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.

2.1.1. 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.

2.1.2. 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.

2.2. Methods

2.2.1. 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).

2.2.2. 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.

2.3. 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.

3. 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) ($p < .001$).