



ANALYSIS OF ASSOCIATION OF -174G/C INTERLEUKIN 6 POLYMORPHISM AND THERAPEUTIC RESPONSE TO METHOTREXATE IN RHEUMATOID ARTHRITIS

ANALIZA POVEZANOSTI 174G/C INTERLEUKIN 6 POLIMORFIZMA I TERAPEUTSKOG ODGOVORA NA METOTREKSAT U REUMATOIDNOM ARTRITISU

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Abstract

Introduction: Rheumatoid arthritis (RA) is a systemic autoimmune disease with IL-6 as a main mediator of systemic and localized inflammation. The most often used drug for the treatment of RA is methotrexate (MTX).

Aim: To explore if there is an association between the 174G/C polymorphism in IL6 gene and response to methotrexate therapy in RA patients.

Materials and Methods: Study has included 131 RA patients treated and followed at the Institute of Rheumatology in Belgrade. Clinical response to the MTX therapy was conducted following the EULAR response criteria. Each patient's DNA was isolated by salting-out method. For each patient IL6 174G/C polymorphism genotype was determined by allele specific PCR method. For statistical analyses we have used SPSS program version 17.0.

Results: According to EULAR response criteria 103 (78.6 %) patients were responders 12 (9.2 %) good and 91 (69.5 %) moderate response) and 28 (21.4 %) were non responders. Among all patients in 47 (35.9 %) we have detected GG genotype, in 69 (52.7 %) GC genotype and in 15 (11.5 %) CC genotype. We have observed no association between genotypes or alleles and response to MTX therapy.

Conclusion: No association of -174G/C IL-6 polymorphism and therapeutic response to MTX in RA patients in Serbian population was observed.

Keywords:

IL-6,
Polymorphism,
Rheumatoid Arthritis,
Methotrexate



Sažetak

Uvod: Reumatoidni artritis (RA) je sistemska autoimuna bolest sa interleukinom 6 (IL-6) kao glavnim posrednikom sistemske i lokalne upale. Najčešće korišćeni lek u terapiji RA je metotreksat (MTX).

Cilj: Cilj ovog rada bio je da se ispita da li postoji povezanost između 174G/C polimorfizma u IL-6 genu i odgovora na terapiju metotreksatom kod pacijenata sa reumatoidnim artritismom.

Materijal i metode: Studija je uključila 131 pacijenta sa RA. Pacijenti su lečeni i praćeni na Institutu za reumatologiju u Beogradu. Klinički odgovor na MTX terapiju procenjivan je na osnovu EULAR kriterijuma. Metodom izolovanja izolovana je DNK svakog bolesnika. Genotip 174G/C polimorfizma IL-6 gena za svakog pacijenta je određivan metodom polimeraznom lančanom reakcijom (PCR) za specifični alel. Za statističke analize koristili smo SPSS program, verziju 17.0.

Rezultati: Na osnovu EULAR kriterijuma, 103 (78,6%) pacijenta su imala odgovor na terapiju (12 (9,2%) dobar i 91 (69,5%) umeren odgovor), a 28 pacijenata (21,4%) nije imalo odgovor na terapiju. Kod 47 (35,9%) pacijenata otkriven je GG genotip, kod 69 (52,7%) GC genotip i kod 15 (11,5%) CC genotip. Nismo uočili povezanost genotipova ili alela i odgovora na terapiju metotreksatom.

Zaključak: Ova studija nije pokazala povezanost -174G/C polimorfizma IL-6 gena i odgovora na MTX kod pacijenata sa reumatoidnim artritismom u našoj populaciji.

Ključne reči:

IL-6,
polimorfizam,
reumatoidni artritis,
metotreksat

Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disease in which one's joints are attacked (1), causing joint inflammation. One of the many mediators that contribute to the pathogenesis of RA and participate in the body's inflammatory response is interleukin-6 (IL-6) (2). It is considered to be a main mediator of systemic and localized inflammation in RA, high levels of it being detected in synovial fluids of inflamed joints (2). Many factors regulate its level, including polymorphisms within the promoter regions of the IL-6 gene. Such a polymorphism is at position -174, comprising of a single nucleotide change from G to C. This -174 G/C biallelic polymorphism has been associated with this cytokine production differences, and thus may have an effect on RA (3,4).

A drug used for the treatment of RA is methotrexate (MTX). It is considered to be the most effective medication for the treatment of RA, due to its ability to interrupt the process that causes inflammation (5). More specifically, it functions to suppress the immune system by blocking the body's use of folic acid. It does this by inhibiting the folic acid reductase, leading to the subsequent inhibition of DNA synthesis (6).

Methotrexate is furthermore considered an anchor drug, to which other DMARDs are added, in pursuit of an optimal therapeutic effect (7). Presently, no reliable tools of predicting the patients' treatment response to MTX have been developed.

Given the lack of studies tackling this correlation, the aim of this study was to investigate the association of 174 G/C interleukin 6 polymorphism and therapeutic response to methotrexate in rheumatoid arthritis. The scope of the investigation was limited to Serbian population.

Material and Methods

Study Design

Patients included in our study were treated and followed at the Institute of Rheumatology in Belgrade, Serbia. The scope of investigation was limited to 131 RA patients whom consented to participating. The protocol of the study was approved by the Ethics Committee of the Institute of Rheumatology. The revised classification criteria for RA of American College of Rheumatology (ACR) from 1987 was used for diagnosis of the disease for each patient (8).

Current or past treatment with MTX for at least six months was set as the main criterion for patient inclusion in the study. Among the allowed treatments undertaken by patients were the following: stable dosages of non-steroidal anti-inflammatory drugs (NSAIDs), low-dose corticosteroids (≤ 10 mg/day), previous disease-modifying antirheumatic drugs excluding MTX (DMARDs - Aurotherapy, Sulphasalazine, Chloroquine) and folic acid supplementation.

To assess the disease activity in each patient we have used the Disease Activity Score (DAS28), calculated from 28 tender and 28 swollen joint counts, the erythrocytes sedimentation rate (ESR) and the patient's general health on a visual analogue scale (GH) (9). At the beginning of the MTX treatment DAS28 is represented as DAS28 0, DAS28 score after six months of therapy was DAS28 1, and Δ DAS28 represented the difference between DAS28 0 and DAS28 1 (Δ DAS28 = DAS28 0 - DAS28 1). Estimation of the clinical response to the MTX therapy was conducted following the EULAR response criteria. The result reflecting $\text{DAS28 } 1 \leq 3.2$ and Δ DAS28 ≥ 1.2 was considered a

good response, $3.2 < DAS28 \leq 5.1$ and $0.6 < \Delta DAS28 \leq 1.2$ (or $\Delta DAS28 > 1.2$ independently of $DAS28$ 1 value) a moderate one, and $0.6 < \Delta DAS28 \leq 1.2$ and $DAS28 > 5.1$ (or $\Delta DAS28 \leq 0.6$ independently of $DAS28$ 1 value) a poor response (10). Using this criterion, patients were classified into “responders” (good and moderate response) and “non-responders”.

Molecular genetic examination

Molecular genetic examination was conducted at the Institute of Human Genetics, Faculty of Medicine University of Belgrade, Serbia.

For the isolation of patients’ DNA, salting-out method was used (11). Allele-Specific Polymerase Chain Reaction (ASPCR) was then used for detection of the 174G/C polymorphism genotypes. In this reaction, three primers were used: a common reverse (Pr) and two forward (Pf). Sequences of these primers are given in **Table 1**.

Table 1. Sequences of primers used for allele specific PCR.

| | | | |
|--------|----|--------------------|----|
| Pf (C) | 5’ | CCCTAGTTGTGCTTGCC | 3’ |
| Pf (G) | 5’ | CCCTAGTTGTGCTTGCG | 3’ |
| Pr | 5’ | GAGCTTCTCTTTCGTTCC | 3’ |

Pf was constructed in two allele-specific forms: 1) Pf (C) and 2) Pf (G), with different tails (C or G at the 3’ end).

Every DNA sample was tested under two reactions, one with the pair of Pf (C) and Pr primers and with the pair of Pf (G) and Pr primers. Expected PCR product was 190 bp long for both reactions. The amplification in both reactions rather than just one provided us with the indication of the heterozygote genotype (CG), instead only for a homozygote (CC or GG).

PCR conditions were: Initial denaturation at 95°C for 5 minutes followed with 40 cycles composed of denaturation at 94°C for 45 sec, hybridization at 60°C for 45 sec and extension at 68°C for 1 min and final extension at 68°C for 5 min. The PCR reaction was undergone with ABI Thermal Cycler 9700.

PCR products were electrophoresed on the 8% polyacrylamide gel stained with SYBR Safe dye.

Statistical analysis

Statistical analysis was performed with the SPSS version 17.0 (SPSS Inc. Chicago, Illinois, USA). Genotypes were examined in connection with numerous factors among the responders and non-responders: differences in patient, disease and treatment characteristics. While the continuous variables were analyzed with the use of either Student’s t-test or Mann-Whitney (depending on the homogeneity of the variable contribution), the discontinuous variables were tackled with the Chi-square or Fisher test.

Results

Out of the 131 individuals participating in the study, a vast majority accounted for females (83.96 %). The mean age was 57.76 ± 10.86 (20- 84). The duration of disease was 49.57 ± 35.24 months (6- 240) and the duration of MTX treatment 37 ± 28.87 months (6- 120). Weekly MTX doses were 10.11 ± 2.75 mg (7.5 mg- 20.0 mg), $DAS28$ at the beginning of the treatment ($DAS28$ 0) 7.71 ± 0.83 (5.53- 9.11), and after 6 months of the MTX therapy ($DAS28$ 1) 5.27 ± 1.60 (1.64- 8.46).

According to the EULAR response criteria, 103 of RA patients (78.6 %) were classified as responders and 28 (21.4 %) as non-responders after MTX therapy of 6 months. Among responders, 12 (9.2 %) were good responders and 91 (69.5 %) moderate responders. Among all patients 66 (50.4 %) received folic acid supplementation and 92 (70.2 %) received corticosteroids. Comparing the responders and non-responders in regards to additional treatments undertaken (folic acid and corticosteroids), there was no statistically significant difference between these two groups although non-responders received corticosteroids more often ($p = 0.061$). Responders received statistically significantly less MTX compared to non-responders ($p = 0.003$). Differences in clinical and demographic characteristics between responders and non-responders are presented in **Table 2**.

In the studied group of patients, GG genotype was detected in 8 non responders and 39 responders (6 good/ 33 moderate), GC genotype in 15 non responders and 54 responders (5 good/ 49 moderate) and CC genotype in 5 non responder and 10 responders (1 good/ 9 moderate). Frequency of allele G was 62.21 %, and allele C was 37.78%. Frequencies of IL-6 -174G/C polymorphism genotypes in RA patients are presented in **Table 3**.

We have observed no differences in genotypes frequencies between responders and non-responders. Additionally, there was no statistically significant difference in response between carriers of different alleles (GG vs. CC/GC, $p = 0.348$, Risk ratio 0.939, CI 95% (0.8295- 1.0639) and CC vs. GG/GC, $p = 0.312$, Risk ratio 1.681, CI 95 % (0.7525- 3.7558)).

Table 2. Differences in clinical and demographic characteristics between responders and non-responders; *- mean ± std. deviation, ** - n, %

| variables | responders | non-responders | p |
|--------------------------------------|-------------|----------------|--------------|
| duration of disease (months)* | 47.53±35.73 | 57.07±32.89 | 0.814 |
| duration of MTX treatment (months) * | 35.50±28.08 | 44.68±31.08 | 0.629 |
| weekly MTX dose (mg) * | 9.66±2.43 | 11.786±3.25 | 0.003 |
| age* | 58.13±11.08 | 56.43±10.06 | 0.255 |
| folic acid** | 54 (52.4) | 12 (42.8) | 0.401 |
| corticosteroids** | 68 (66.0) | 24 (85.7) | 0.061 |

Table 3. Frequencies of IL-6 -174G/C polymorphism genotypes in RA patients

| Genotype | All, n (%) | Responders, n (%) | Non-responders, n (%) | p |
|----------|-------------|-------------------|-----------------------|-------|
| GG | 47 (35.9) | 39 (82.9) | 8 (17.0) | 0.404 |
| CC | 15 (11.5) | 10 (66.73) | 5 (33.3) | |
| GC | 69 (52.7) | 54 (78.3) | 15 (21.7) | |
| Total | 131 (100.0) | 103 (78.6) | 28 (21.4) | |

Discussion

As mentioned in the introduction, IL-6 has a crucial role in the pathogenesis of RA and the polymorphisms in the gene coding for this molecule should affect the disease activity. Higher frequencies of IL-6 -174G/C polymorphism CC and CG genotypes have been observed in RA patients compared to control group (12). Nevertheless, contrary results were found by You CG et al. (13).

It has been shown that the individual differences in the polymorphic cytokine genes can lead to individual variation in release of cytokines after treatment with methotrexate (14). Furthermore, there have been studies considering polymorphisms in genes coding for cytokines other than IL6 that showed influence of this polymorphisms on therapy response in RA patients (15).

Consequently, we have assumed that different expression of the IL6 gene, dependent on the 174G/C polymorphism genotypes, may influence patients' response to MTX therapy; however, our study on 131 RA patients found no such association. In agreement with our results, in a clinical study conducted on Mexican population, RA Ruiz-Padilla A. J. et al. 2016 concluded that among patients receiving MTX therapy IL6 174G/C genotypes were not predictor of therapeutic failure (16).

In conclusion, this study observed no significant association of -174G/C IL-6 polymorphism and therapeutic response to MTX in RA in Serbian population. Among the possible reasons behind such results could be a small number of patients included, together with a small dose of MTX.

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