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Original article

Association of the A Allele of the TNF-Alpha-308 G/A Gene Polymorphism with Radiographic Progression in Rheumatoid Arthritis

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SUMMARY

To examine whether the presence of the rare A allele of the TNF-Alpha-308 G/A gene polymorphism is associated with erosive arthritis and rapid radiological progression of the disease.

The examined group included 131 patients with early RA. Using the PCR-RFLP method, the TNF-Alpha-308 G/A gene polymorphism was determined for all patients. In relation to the presence of the A allele of the examined polymorphism, the patients were divided into two subgroups: subgroup A (G/A and A/A genotypes) and subgroup G (G/G genotype). Based on the presence of the destructive changes in joints found in the initial radiographs, the findings were classified as erosive and non-erosive RA. Radiological progression was assessed on the basis of the annual change in the Larsen score – LS (0-200).

Group A comprised 62 (47.33%) patients, while group G comprised 69 (52.67%) patients. The presence of cysts and erosions in subgroups A and G was compared before the start of the methotrexate therapy. It was determined that erosions (erosive RA) were statistically significantly more frequent in group A (83.87% of patients) in comparison with group G (44.93% of patients), $p < 0,001$. Group A patients had a higher value of the Larsen score in relation to the group G both before and after methotrexate was administered for a year (before therapy, LS in group A: 48.58 ± 20.54; in group G 20.73 ± 12.31; $p < 0.01$; after MTX therapy, LS in group A: 58.32 ± 22.25; in group G 25.35 ± 14.57; $p < 0.001$). The average value of the annual LS change in group A was statistically significantly greater than the change in group G patients (9.98 ± 4.95 to 5.23 ± 2.72) ($p < 0.05$).

The presence of the A allele of the TNF-Alpha-308 G/A gene polymorphism is associated with the occurrence of early erosive joint changes and more rapid radiological progression of the disease.

Key words: **TNF gene polymorphism, radiographic progression, rheumatoid arthritis**

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INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease, caused by an autoimmune destruction of synovial joints, present in around 1% of the Caucasian population. If RA takes an evolutive course and is treated inadequately, it can lead to substantial disability and premature mortality.

Even though the pathogenesis of the disease has not been fully clarified yet, it is known that the tumour necrosis factor alpha (TNF-α) plays a key role in the inflammatory process of this most common autoimmune arthropathy. Its role in joint destruction has been proven by simulating the release of matrix metallo-proteinases and other proteolytic enzymes which degrade the components of the extracellular matrix (1). It has also been shown that the activation of osteoclasts through TNF-α is indirect and a consequence of the induction of the RANK/RANKL signalling pathway. Rheumatoid joint synovium is environmentally suitable for the differentiation of osteoclasts since it is rich in macrophages which express RANK and which can be differentiated into osteoclasts (2).

RA differs from other inflammatory arthropathies in its pronounced tendency towards destructive changes in joints. The degree of joint damage represents an important prognostic parameter for the ultimate outcome of the disease. Joint destruction (cysts and erosions) is an early occurrence in RA. It is believed that erosions appear in 10-26% of patients in the first three months of the disease, in 60% of patients before the end of the first year, and in up to 75% of patients in the first two years from the onset of the disease. Progression of destructive changes in joints is most rapid in the first years of the disease. It has been shown that joint damage is greater in the first two years of the duration of arthritis than in all of the remaining years collectively (3).

Cartilage and bone damage can be visible in classic radiographic imaging and quantified by using various scores, as the total score of joint damage. The first radiological score systems were described more than 40 years ago (4). The most often used ones are Sharp van der Heijde and Larsen scores (5) with different modifications. Even though radiology is still the cornerstone for the detection and monitoring of joint damage, this method has low sensitivity in discovering early erosions and assessing synovitis. Recently, with the aim of establishing early diagnosis of RA, rheumatologists have been increasingly "dependent" on new imaging techniques, above all joint MR (magnetic resonance) and US (ultrasound).

Numerous studies have examined the prediction factors of joint damage in early RA. The factors that have been confirmed as influential to the degree of joint destruction are the composite index DAS 28 (SE), the level of CRP, the level of rheumatoid factor (RF), the level of anti-citrullinated protein antibodies (ACPAs), and the initial level of joint damage. New predictors of joint damage are the markers of cartilage and bone decomposition – C-terminal telopeptide I and II (CTX-I and CTX-II) and RANKL/OPG (6).

The results of the studies that deal with the association of the TNF-α gene polymorphism with radiographic progression of the disease are varied. On the one hand, certain authors point to the existence of the association of -308 G/A polymorphism with a higher degree of joint destruction in patients with RA (7). On the other hand, the results obtained by other authors do not show any effect of -308 G/A polymorphism on the activity of the disease, a more severe course or prognosis of RA (8).

AIMS

To examine whether the presence of the rare A allele of the TNF-Alpha-308 G/A gene polymorphism is associated with erosive arthritis and rapid radiological progression of the disease.

EXAMINEES AND METHODS

The research was conducted at the Clinic for Rheumatology of the Institute for Treatment and Rehabilitation "Niška Banja" and the Scientific Research Centre for Biomedicine of the Faculty of Medicine, University of Niš. In total, 131 subjects with early RA were involved in this prospective study. RA was diagnosed based on ACR/EULAR 2010 criteria (9). All examinees were informed of the aims of the study beforehand, and they confirmed their approval to participate in the study by signing an informative consent agreement. The Ethical Committee of the Faculty of Medicine in Niš also provided its assent for this research (decision No. 01-206-6). The research was further approved by the Ethical Committee of the Institute for Treatment and Rehabilitation "Niška Banja" (decision no. 03-7974).

The average age of the examinees was 55.63 ± 11.46 years (95% CI 53.64-57.61). Their age ranged from 24 to 77 years. The examined group comprised 23 (17.56%) men and 108 (82.44%) women.

The isolation of DNA from the examinees' blood was performed using the commercial QIA amp DNA Blood Mini isolation kit (Quiagen, GmbH, Hilden, Germany), after which the obtained samples were tested for the TNF-Alpha-308 G/A gene polymorphism, by applying the polymerase chain reaction – the restriction fragment length polymorphism (PCR-RFLP) method (10). PCR amplification was checked using agarose gel electrophoresis (2%). After the agarose gel confirmation, amplification fragments were sliced into smaller ones, depending on the presence of the reaction spot polymerphism, using the restriction digest technique with the restriction endonuclease, Ncol enzyme (Fermentas, GmbH, St. Leon-Rot, Germany). The obtained DNA fragments were detected using the vertical 8% polyacrylamide gel electrophoresis, after which DNA fragments were visualized under UV rays. Blood samples for DNA analysis were taken during routine laboratory examination of patients. The examination was conducted at the Scientific Research Centre for Biomedicine of the Faculty of Medicine in Niš.

With the aim of diagnosing and monitoring radiological progression of arthritis, all patients were subjected to standard radiography of the hands and feet (anteroposterior projection) at the start of the research and after a year of monitoring. Based on the radiological presentation of the disease in the hands and feet, the radiological findings were qualified as non-erosive and erosive arthritis. Radiological progression of patients treated by methotrexate was assessed on the basis of the change in the Larsen score which quantifies the degree of joint damage. Values of the modified Larsen score (11) ranged from 0 to 200. Thirty-two joints were analyzed in the hands and feet radiographs (8 PIP of hands, 2 IP of thumb joints, 10 MCP, 2 RC, 2 IP of big toes, 8 MTP). The gradation of changes in joints was performed after a comparison with standard reference films: 0^{th} degree – intact joint space, 1^{st} degree – soft tissue swelling, joint space narrowing, periarticular osteoporosis, 2^{nd} degree – erosions with joint surface destruction of less than 25%, 3^{rd} degree – joint surface erosion of 26-50%, 4^{th} degree – joint surface erosion of 51-75%, 5^{th} degree – erosions with joint surface destruction of more than 75% (5). Before the administration of methotrexate therapy, all patients were subjected to standard radiography of the lungs and heart (anteroposterior projection).

Input, tabular and graphic presentation of the data was performed using the MS Office Excel program, while statistical calculations were carried out in the SPSS package, version 15.0. The results of the statistical analysis are presented both in the tabular and graphic form.

RESULTS

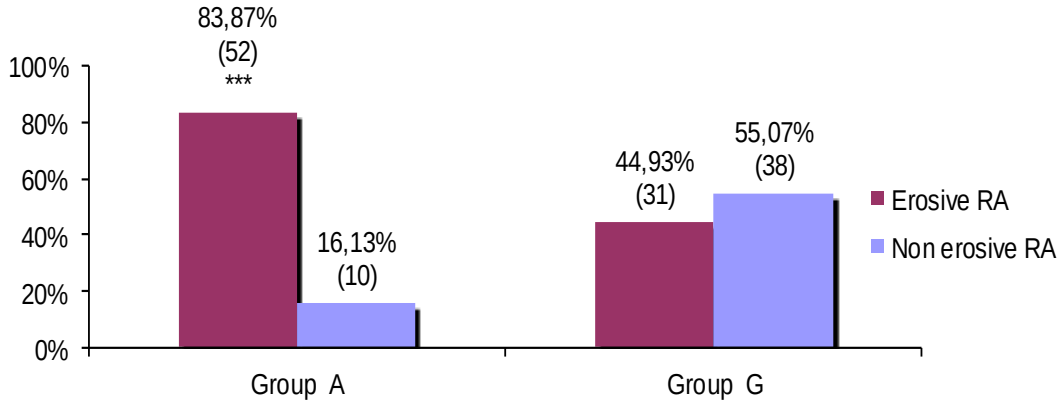
The average duration of disease symptoms before the diagnosis was 9.27 ± 2.67 months. All patients suffered from moderately or highly active RA, with the majority of them (117 – 89.31%) experiencing signs of the highly active disease (DAS 28 SE > 5.1). The average DAS 28 (SE) was 6.07 ± 1.23. Increased values of C-reactive protein (CRP) were present in 63.35% of patients. More than two thirds of patients were RF positive (69.46%). Out of the total number of examinees, 80.15% possessed anti-citrullinated protein antibodies (ACPAs). The majority of patients began the research with radiologically registered destructive changes in joints (63.35%). The average dosage of methotrexate at the start of the examination was 15.75 mg per week.

On the basis of the presence of the A allele of the TNF-Alpha-308 G/A gene polymorphism, examinees were divided into two subgroups: group A – with the A allele in their genotype (G/A and A/A genotypes) consisting of 62 examinees (47.33% of the total number of examinees), and group G – without the A allele, with the G/G (wild type) genotype, which consisted of 69 examinees (52.67%).

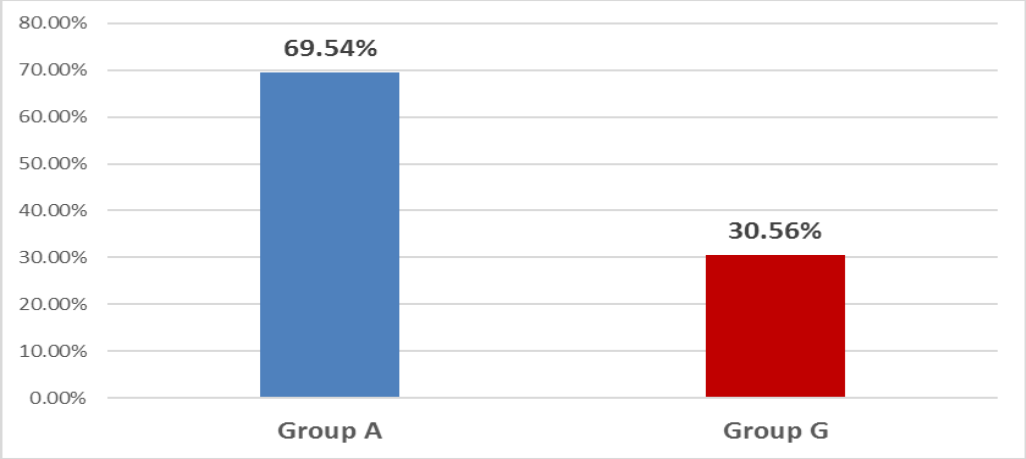
There are no statistically significant differences between groups A and G regarding age and sex. Based on the variation coefficient, it is evident that both subgroups are homogeneous in terms of age. The average duration of disease in subgroups A and G doesn't show a statistically significant difference: in group A, the average duration of RA before the beginning of treatment was 10.3 ± 2.1 months, while in group G it was 8.7 ± 1.9 months.

The presence of cysts and erosions (destructive joint changes) was determined in the radiography of the hands and feet in both subgroups of RA patients. The data on the distribution of joint damage at the beginning of the research in subgroups A and G are given in Graph 1. Pearson's χ 2 test determined that erosions were statistically more significantly present in group A in relation to group G (83.87% to 44.93%) (Graph 1).

In the group of patients with non-erosive arthritis, after a year of monitoring through control radiography of the hands and feet, the occurrence of cystic and erosive changes in joints was analyzed. Out of the 48 cases of initially non-erosive arthritis, 36 (75%) showed the destruction of cartilage and bone after a year. Among them, 25 (69.44%) were carriers of the A allele in the position of the TNF-α-308 gene, significantly more than in the group of RA patients with registered joint progression but without the A allele of the examined polymorphism (Graph 2).



Graph 1. Destructive changes in joints in the examined subgroups of the RA group



Graph 2. Radiographic progression in joints in relation to the presence of the A allele

Using the Larsen score, the degree of the destructive joint changes was quantitatively assessed for group A and G patients, both before and after a year of methotrexate treatment. Group A patients possessed significantly higher Larsen score values at the beginning and after 12 months of therapy (Table 1).

Table 1. Larsen score in patients treated with methotrexate in relation to the presence of the A allele

Group	Group A (N=62) X±SD	Group G (N=69) X±SD	p
Before MTX th	**45.58 ± 20.54**	**20.73 ± 12.31**	**<0.01**
After MTX th	**58.32 ± 22.25**	**25.35 ± 14.57**	**<0.001**

MTX –methotrexate, SD - standard deviation

With the aim of monitoring the radiological progression of the disease, after a year of methotrexate treatment, control radiography of the hands and feet was performed. The difference between the initial values of the Larsen score and the Larsen score after a year of methotrexate therapy was used to assess the radiological progression of the disease.

Group A patients showed a statistically significantly higher average value of the annual change in the Larsen score in comparison with the change in the Larsen score of group G patients (9.98 ± 4.95 to 5.23 ± 2.72) (Table 2).

Table 2. Radiographic progression in patients treated with methotrexate in relation to the presence of the A allele

Group	Group A (N=62) X±SD	Group G (N=69) X±SD	p
Annual change in LS	**9.98 ± 4.95**	**5.23 ± 2.72**	**< 0.05**

LS - Larsen score, SD - standard deviation

DISCUSSION

Persistent synovial inflammation is the precondition for the appearance of joint destruction in most cases. The examined TNF-α cytokine acts as an intermediary in both processes (inflammation and joint destruction). The probable mechanism of TNF-α activity in the process of bone and cartilage destruction is the stimulation of osteoblast apoptosis and the activation of osteoclasts (11). However, not all RA patients display the association of inflammation with the destructive changes in joints, which indirectly implies the existence of other individual factors that influence these two independent aspects of the disease. Certain authors suggest that there are genes which regulate the level of inflammation and genes which are responsible for the process of bone resorption.

Despite new sensitive diagnostic procedures, conventional radiography is still standard in everyday clinical practice for the assessment and monitoring of joint damage in RA. The analysis of the radiography of the hands and feet was performed to assess joint destruction in RA patients. At the beginning of the examination, the majority of patients already showed the signs of erosive arthritis (83 examinees – 63.36%). The abundance of early erosive changes in joints in the examined group is in accordance with the literature (12) and well expected, bearing in mind that the examined group in this research comprised patients with highly active RA. One of the possible reasons behind such a strong presence of erosive arthritis in this research lies in the fact that the average duration of the disease before diagnosis and first radiography was more than 9 months (longer than the other studies with early RA) (3, 13). By examining the influence of the presence of the A allele of the TNF-Alpha-308 G/A gene polymorphism on bone and cartilage destruction in the early course of the disease, it was determined that erosions were more present in the A allele carriers than in the G/G genotype. In group A, 83.37% of patients suffered from early destructive changes in joints, which was significantly greater than group G where radiologically proven joint changes were registered in 44.93% of patients. These results are in agreement with the results of a group of Portuguese rheumatologists who, by monitoring 554 patients with the duration of RA of up to 10 years, showed the association of -308 G/A gene polymorphism with the high Sharp van der Heidje score of radiological damage (14). Another confirmation of such results lies in the observation of Nemec et al. who emphasized this polymorphism as an important predictor of early joint destruction (7).

The probable mechanism of TNF-α activity on development joint destruction is the effect on the activity of proteolytic enzymes. Since it is previously shown that TNF–α -308A allele is associated with an increased production of TNF–α (15) and that MMP–9 production correlates with TNF–α level in plasma (16), we analyzed the impact of TNF–α -308A allele on MMP–9 activity in blood plasma and synovial fluid of RA patients. The obtained results showed that MMP–9 activity is higher in blood plasma and especially in synovial fluid of the patients who were carriers of genotypes that contain TNF–α -308A allele (GA and AA) (17).

Knowing the factors that influence the rate of joint damage progression is of utmost importance in every day clinical practice since it can determine the concept of RA treatment. Previous studies have corroborated the predictive importance of age, sex, CRP level, SE rate and size of affected joints in relation to the degree of joint damage (7, 8). Our previous papers showed that the greatest prognostic importance for the degree of joint damage after four years of RA duration lies in the value of the Larsen score at the onset of the disease (18). The results of the present study affirm the examined -308 G/A polymorphism, i.e. the presence of the A allele, as a new prognostic marker for radiological progression of RA. The presence of the A allele of the examined polymorphism indicates that the group of patients with early RA should be subjected to early aggressive therapy using medication which influences the course of the disease, as well as early administration of biological medication which also affects the course of the disease. Such a therapeutic approach will cause joint destruction to slow down and allow a proper control of inflammation and better prognosis of the disease.

CONCLUSION

The presence of the A allele of the TNF-Alpha-308 G/A gene polymorphism in RA patients is associated with the development of early erosive joint changes and rapid radiological progression of the disease.

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Udruženost A alela polimorfizma -308 G/A gena za TNF alfa sa radiografskom progresijom reumatoidnog artritisa

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SAŽETAK

Cilj rada bio je ispitati da li je prisustvo retkog A alela polimorfizma -308 G/A gena za TNF alfa udruženo sa erozivnim artritisom i brzom radiološkom progresijom bolesti.

Ispitivanu grupu činio je 131 bolesnik sa ranim reumatoidnim artritisom (RA). Svim bolesnicima je metodom PCR-RFLP određivan polimorfizam -308 G/A gena za TNF alfa. U odnosu na prisustvo A alela ispitivanog polimorfizma, bolesnici su podeljeni u dve podgrupe: podgrupu A (genotipovi G/A i A/A) i podgrupu G (G/G genotip). Na osnovu prisustva destruktivnih zglobnih promena na početnim radiografijama nalazi su kvalifikovani kao erozivni i neerozivni RA. Radiološka progresija procenjivana je na osnovu godišnje promene Larsen skora – LS (0-200).

Grupi A pripadala su 62 (47,33%) bolesnika, G grupi 69 (52,67%) bolesnika. Grupe su bile homogene po starosnoj i polnoj distribuciji. Komparirali smo prisustvo cisti i erozija u A i G podgrupi pre početka terapije Metotreksatom. Utvrdili smo da su erozije (erozivni RA) statistički značajno češće u grupi A (83,87% bolesnika) u odnosu na G grupu (44,93% bolesnika), $p < 0,001$. Veću vrednost Larsen skora imali su bolesnici A grupe u odnosu na G grupu kako pre tako i nakon godinu dana od primene Metotreksata (pre th LS u A grupi: 48,58 ± 20,54; u G grupi 20,73 ± 12,31; $p < 0,01$, posle th MTX-om LS u A grupi 58,32 ± 22,25; u G grupi 25,35 ± 14,57; $p < 0,001$).

U A grupi prosečna vrednost godišnje promene LS statistički je značajno veća u odnosu na promenu LS kod bolesnika G grupe (9,98 ± 4,95 prema 5,23 ± 2,72) ($p < 0,05$).

Prisustvo A alela polimorfizma -308 G/A gena za TNF alfa udruženo je sa pojavom ranih erozivnih zglobnih promena i bržom radiološkom progresijom bolesti.

Ključne reči: **polimorfizam -308 G/A gena za TNF alfa, radiografska progresija, reumatoidni artritis**