DOI: 10.4274/tftr.56.182

Assessment of Serum Sialic Acid and Oxidative Stress Parameters in Rheumatoid Arthritis Patients Receiving Anti-TNF-α and Conventional Therapies

Anti-TNF-α ve Geleneksel Tedavi Alan Romatoid Artritli Hastalarda Serum Sialik Asit ve Oksidatif Stres Parametrelerinin Değerlendirilmesi

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Summary

Objective: In this study, we aimed to evaluate sialic acid (SA) levels and oxidative stress parameters by measuring nitric oxide metabolites (NOx), thiobarbituric acid-reactive substances (TBARS) and thiol (SH) in rheumatoid arthritis (RA) patients treated with either conventional or anti-tumor necrosis factor-α (anti-TNF-α) therapy.

Materials and Methods: Eighty-six RA patients and 20 healthy controls were enrolled in the study. All RA patients fulfilled the American College of Rheumatology (ACR) criteria for RA. Thirty patients were on anti-TNF-α therapy. The rest of the patients were either treated with two or three disease-modifying anti-rheumatic drugs. Health Assessment Questionnaire (HAQ) score and the Disease Activity Score 28 (DAS28) were calculated in all patients. NOx, TBARS, SA, and SH levels were measured in both patients and controls.

Results: The SA levels were statistically higher in RA patients than in controls. We found increased levels of TBARS and NOx and decreased levels of SH in both groups, but these results were statistically insignificant. Spearman correlation analysis revealed positive correlation between serum TBARS levels and both NOx (r=0.322, p=0.001) and SA (r=0.242, p=0.017) levels.

Conclusion: Our results confirm the role of SA in the pathogenesis of RA and its correlation with oxidative stress. Further studies on larger numbers of subjects and with longer treatment durations are needed to analyze the long-term effects of RA therapies on serum oxidative stress parameters. *Turk J Phys Med Rehab 2010;56:182-5.*

Key Words: Anti-TNF-α, treatment, oxidative stress, rheumatoid arthritis, sialic acid

Özet

Amaç: Bu çalışmada geleneksel veya anti-TNF-α tedavisi alan romatoid artrit (RA) hastalarında nitrik oksit metabolitleri (NO), tiobarbitürik asit türevi aktif maddeler (TBARS) ve tiyol grupları (SH) ölçümü ile oksidatif stres ve sialik asit (SA) düzeylerini değerlendirmeyi amaçladık.

Gereç ve Yöntem: Çalışmaya 86 RA hastası ile 20 sağlıklı kontrol dahil edildi. RA hastalarının tümü, RA için ACR kriterlerini karşılamaktaydı. Otuz hasta anti-TNF-α tedavisi alıyordu. Diğer hastalar hastalık düzenleyici anti-romatizmal ilaçlardan iki veya üçünü kullanıyordu. Tüm hastaların HAQ ve DAS28 skorları hesaplandı. Hem hastaların hem de kontrollerin NOx, TBARS, SA ve SH düzeyleri ölçüldü.

Bulgular: RA hastalarının SA düzeyleri kontrol grubuna göre istatistiksel olarak anlamlı derecede yüksekti. Her iki grupta da TBARS ve NOx düzeylerinin artmış, SH düzeylerinin azalmış olduğunu bulduk. Ancak bu bulgular istatistiksel anlamlılığa ulaşmıyordu. Spearman korelasyon analizi sonucunda serum TABRS düzeyleri ile hem NOx hem de SA düzeyleri arasında olumlu korelasyon olduğu ortaya çıktı (r=0,322, p=0,001) and SA (r=0,242, p=0,017).

Sonuç: Bulgularımız SA'nın RA patogenezindeki rolünü ve oksidatif stresle ilişkisini desteklemektedir. RA tedavisinin serum oksidatif stres parametrelerine uzun dönem etkisini incelemeyi hedefleyen, daha geniş sayıda hastayla ve daha uzun süreli tedavi takibiyle yapılacak çalışmalara ihtiyaç bulunmaktadır. *Türk Fiz Tıp Rehab Derg 2010;56:182-5.*

Anahtar Kelimeler: Anti-TNF-α, tedavi, oksidatif stres, romatoid artrit, sialik asit

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Phone: +90 380 541 41 07 E-mail: drselmayazici@yahoo.com.tr **Received/Geliş Tarihi:** December/Aralık 2009 **Accepted/Kabul Tarihi:** May/Mayıs 2010

Introduction

Rheumatoid arthritis (RA), a chronic autoimmune disorder affecting about 1% of the adult population, is associated with synovial proliferation and excessive mononuclear infiltration leading to the development of cartilage and subchondral bone erosions with unclarified etiopathogenesis (1). The oxidative stress has an important role in the development of proliferative synovitis and destruction (2-4). Besides, in the inflamed joints of RA cases, there is an increased production of many cytokines, including tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) (5,6). It has been shown that elevated TNF-α levels increase the production of endogenous reactive oxygen species (ROS), which contribute to oxidative stress. ROS act as secondary messengers to stimulate nuclear factor kappa B-dependent expression of pro-inflammatory cytokines and form an amplification loop, which feeds back to further excite the production of additional ROS (7).

Sialic acid (SA) is a terminal component of the nonreducing end of carbohydrate chains of glycoproteins and glycolipids (8). Serum or plasma SA concentration is a marker of acute-phase response, since many of the acute-phase proteins (e.g., α1-acid glycoprotein, fibrinogen, and haptoglobin) are glycoproteins including SA terminal sugar of the oligosaccharide chain (9). During inflammatory processes, increased SA concentrations have been reported. Elevated SA levels have also been reported as a strong predictor of cardiovascular mortality in the general population (10-12). Although it has been shown that atherosclerotic disease is increased in RA, the cause of this acceleration of atherosclerosis has not yet been fully clarified (13). Serum SA may have a role in this increased risk of atherosclerosis.

The control of inflammation may have influence on the acute-phase response. In the literature, there is limited information about the effect of treatment modalities on the levels of SA and oxidative stress parameters in RA patients.

In this study, we aimed to evaluate SA levels and oxidative stress parameters by measuring nitric oxide metabolites (NOx), thiobarbituric acid-reactive substances (TBARS) and thiol (SH) in RA patients treated with either conventional or anti-TNF-α therapy.

Materials and Methods

Patients: We enrolled 86 RA patients and 20 healthy controls into the study. All patients fulfilled the American College of Rheumatology 1987 (1987 ACR) criteria for rheumatoid arthritis (14). Thirty-two patients were on anti-TNF-α therapy. The rest of the patients were either treated with two or three disease-modifying anti-rheumatic drugs (DMARDs). Disease activity was measured by the 28-joint count, Disease Activity Score (DAS28) and the functional status was calculated by the Health Assessment Questionnaire (HAQ) in all patients (15,16).

Treatments of both groups are shown on Table 1. Among anti-TNF-α treatment groups, twenty-three patients had received subcutaneous injections of etanercept (25 mg) twice weekly, five patients had received 5 mg/kg infliximab i.v. at weeks 0.2 and 6 and then every 6-8 weeks, four patients had received 40 mg subcutaneous injections of adalimumab every 2 weeks at a dose of 40 mg.

In the conventional treatment group, patients were using double or triple combinations of methotrexate (7.5-20 mg/week), sulfasalazine (2-3 gr/day), hydroxychloroquine (200-400 mg/day) and leflunomide (10-20 mg/day). Additionally, all patients were taking prednisolone 2.5-7.5 mg/day and patients on methotrexate also received folic acid supplementation.

The characteristics of patients are shown in Table 2. All of the patients were informed and the study was approved by Local Ethics Committee of Ankara Training and Research Hospital

Biochemical Analysis: Serum samples were from venous blood and were immediately separated. Erythrocyte sedimentation rate (ESR), levels of C-reactive protein (CRP), hemoglobin and albumin as well as leucocyte (WBC) and platelet counts were measured on the day of sampling. For NOx, TBARS, SH and SA measurements, serum samples were frozen at -80 ºC until assayed. Hemoglobin, platelet and WBC were analyzed with a Coulter LH 750 hematology analyzer (Beckman Coulter Inc., Fullerton, CA, USA). Total nitrite was measured with a modified Griess method. Briefly, nitrate was reduced to nitrite by nitrate reductase and the formed color was measured at the end of the reaction of nitrite with Griess reagent. The resultant color changes were read at 540 nm. Calibration curves were made with sodium nitrite standards prepared in deionized water (17). Serum TBARS levels were determined by the method described by Wasowicz (18). After the reaction of thiobarbituric acid with MDA, the reaction product was extracted in butanol and was measured spectrofluorometrically at wavelengths of 525 nm for excitation and 547 nm for emission. 0-25 μmol/L 1, 1', 3, 3'-tetraethoxypropane solutions were used as standard. SH groups were measured by a colorimetric method, in which SH groups give a chromogen compound with dithiobisnitrobenzoic acid in alkaline pH (19). SH concentrations were calculated by the use of molar absorptivity of reaction product, thionitrobenzoic acid (ε_{412}= 13.600). The resorcinol method was used for SA determination (20). In this method, SA was first dissociated with acid hydrolysis, then resorcinol and Cu2+ were used to obtain chromophore. Finally, chromophore was extracted with n-butanol and read at 580 nm.

Statistical Analysis: Results were presented as mean±SD, with a p-value less than 0.05 indicating statistical significance. The Mann-Whitney U test was used for the comparison of patient and control groups. Spearman correlation analysis was performed for the correlation of the variables. All statistical calculations were analyzed using SPSS version 10.0 for Windows (SPSS Inc. Headquarters, Chicago, Illinois, US).

Results

The study groups comprised 54 RA patients (43 female, mean age: 49 years) treated either with two or three DMARDs, 32 RA patients (24 female, mean age: 46 years) receiving anti-TNF-α treatment and 20 healthy controls (10 female, mean age: 48 years). Demographic features, clinical activity scores and acute phase levels are shown in Table 1. There was no difference between the conventional therapy group and anti-TNF-α treatment group according to age, disease duration and acute-phase levels. The median time from onset to initiation of anti-TNF-α treatment was 16 months, with a range of 8 to 49 months. DAS28 and HAQ score were similar in the conventional and anti-TNF-α therapy groups.

The results are shown in Table 3. The SA levels in RA patients were statistically higher than those in the control group, regardless of the treatment (p=0.001). We found increased levels of TBARS and NOx, and on the other hand, decreased levels of SH in both groups, but these results were statistically insignificant.

Spearman correlation analysis for the variables revealed positive correlation between serum TBARS levels and both NOx (r=0.322, p=0.001) and SA (r=0.242, p=0.017) levels.

There was no correlation between NO metabolites and both the HAQ score and DAS28 in both groups.

Discussion

In this study, SA levels in RA patients were found higher than in healthy controls regardless of the anti-TNF-α treatment used, but were similar in the anti-TNF-α and conventional therapy groups.

ROS are highly reactive molecules that can attack almost every cell component leading to further tissue damage. Cell damage caused by ROS production and lipid peroxidation plays a role in the pathogenesis of a number of acute and chronic diseases, such as inflammation, cancer, liver injury, atherosclerosis and RA (21,22). Several reports have suggested that the increased ROS production in patients with RA causes cell injury (23,24). Malondialdehyde (MDA) is one of the well-known secondary products of lipid peroxidation after exposure to ROS and free radicals and may be used to evaluate oxidative damage by measuring serum TBARS levels (25,26). Plasma SHs are physiological free-radical scavengers and may serve an antioxidant function by several mechanisms. It has been reported that the measurement of plasma total SH is a good reflection of excess free-radical generation both in physiological and pathological conditions in humans (27,28). Nitric oxide (NO), which is synthesized mainly in the endothelium, is a labile molecule with a half-life of only a few seconds. NO is rapidly oxidized by tissue oxygen to stable end-products nitrate (NO3-) and nitrite (NO2). The best index for overall NO production is the total concentration of both nitrate and nitrite (29). Simultaneous release of NO and superoxide anions produce peroxynitrite anion, which is a strong biological oxidant known to oxidize lipids, proteins, and SH groups particularly (30). MDA, total SH and NOx may together give information about the oxidative and nitrosative status of an individual.

Although in this study we found increased levels of NOx, TBARS and decreased levels of SH in RA patients compared to controls, these data were statistically insignificant. Several mechanisms have been proposed for the beneficial effects of corticosteroids and methotrexate in RA patients, one of which is the inhibitory effect of those drugs on phospholipase A2, an enzyme that is known to facilitate the ROS production (31,32). Since our patients were treated with conventional therapies, treatments may have beneficial effects by protecting from ROS damage.

We used TBARS as an indicator of oxidative stress. TBARS showed positive correlations with NOx and SA. These correlations support the idea that there is a relationship between oxidative stress and SA. These two factors are known to have roles in the pathogenesis of atherosclerosis and the correlation between them may have importance in clinical practice. The antioxidant status in RA has been examined before, but to our knowledge, this is one of the first studies that determine the effect of different treatment modalities (conventional and anti-TNF-α therapies) on serum SA and oxidative stress parameters in RA patients.

We did not observe any significant difference between the treatment protocols. By matching the treatment groups in terms of disease activity and acute-phase responses, we hoped to explore whether any differences in the levels of SA and oxidative stress parameters would occur with regard to treatment. It is possible that the small size of the study may have influenced this result. However, we could not find any beneficial changes in the serum oxidative stress parameters and SA relevant to the anti-inflammatory effects of anti-TNF-α therapy. We speculate that

Table 1. Demographic features and laboratory findings of RA patients.

	Anti-TNF- α group n=32	CT group n=54
Age (year)	43.7±11.5	46.9±10.6
Sex (Female/Male)	25/7	43/11
Disease duration (year)	11.1±8.3	10.1±9.8
DAS 28	3.1±1.04	2.9›1.19
HAQ	0.68±0.64	0.46±0.57
ESR (mm/h)	22.2±7.6	19.8±6.4
CRP (mg/L)	1.1 (0.1-7.3)	0.9 (0.1-4.9)
Leucocyte (mm^3)	7550±1570	6930±1990
Platelets (mm^3)	290000±61000	246000±52000
Hemoglobin (g/dL)	12.3±1.7	11±3.3

CT: Conventional treatment, DAS28: Disease Activity Score 28, HAQ: Health Assessment Questionnaire , ESR: Erythrocyte sedimentation rate, CRP: C-reactive protein.

Table 2. Medications of patients.

	Anti-TNF-α group n=32	CT group n=54
Methotrexate (MTX)	20	42
Sulfasalazine (SZP)	3	44
Hydroxychloroquine (HCQ)	3	49
Leflunomide (LEF)	3	8
MTX + INF	4	-
SZP + HCQ + ETA	3	-
MTX + ETA	12	-
MTX + LEF + ETA	1	-
MTX + ADA	3	-
LEF + ADA	1	-
LEF + INF	1	-
ETA (monotherapy)	7	-

CT: Conventional treatment, INF: Infliximab, ETA: Etanercept, ADA: Adalimumab.

Table 3. Median sialic acid and oxidative stress parameters of groups.

	Healthy Control	Conventional treatment	Anti-TNF-α treatment	p
TBARS (μmol/L)	0.74	0.78	0.72	NS
SH (μmol/L)	470	454	447	NS
NOx (μmol/L)	24.2	25.4	26.4	NS
SA (μmol/L)	1.08	1.23a	1.23a	0.001

the biological effects of anti-TNF-α therapies may last longer than the half-life of the drug and, to analyze the long-term effects of these therapies, we need further studies with larger numbers of RA patients and longer treatment durations.

In conclusion, our results confirm the role of SA in the pathogenesis of RA and its correlation with oxidative stress. Further studies are needed to discuss whether SA and oxidative stress markers may be useful in the monitoring of treatment in RA patients.

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