The Relationship Between Disease Activity, Vegf, E-selectin Levels and Arterial Stiffness in Patients with Rheumatic Diseases

Romatizmal Hastalığı Bulunan Hastalarda Arteriyel Sertlik, Vegf ve E-selektin Düzeyi ile Hastalık Aktivitesi Arasındaki İlişki

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ABSTRACT

Introduction: The endothelial damage, changes in vascular permeability and plaque formation are caused by the effects of cytokines and chemokines that plays role in chronic inflammation in rheumatoid arthritis (RA) and ankylosing spondylitis (AS). It has been reported that arterial stiffness and the level of cell adhesion molecules are affected by inflammation. In this study, we aimed to determine the relationship between vascular endothelial growth factor (VEGF), E-selectin, arterial stiffness and disease activity in patients with rheumatic diseases.

Methods: Thirteen patients diagnosed with AS, 28 patients diagnosed with RA and 30 healthy controls were included in the study. Arterial stiffness, VEGF, E-selectin, erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) were evaluated in all patients and healthy controls. Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) and Disease Activity Score-28 (DAS-28) were calculated in patients with rheumatic diseases. The level of serum VEGF and E-selectin were determined by ELISA, and arterial stiffness was measured by oscillometric method. The data were statistically evaluated by using Student t-test, Mann-Whitney U Test and Wilcoxon test.

Results: DAS-28, BASDAI, ESR and CRP levels were significantly decreased on the 3rd month of treatment in all patients (p<0.001). VEGF and E-selectin levels increased, and pulse wave velocity (PWV) and augmentation index (Alx) parameters decreased with treatment. While the level of PWV did not change, Alx decreased on the 3rd month of non- tumor necrosis factor treatment in RA patients.

Conclusion: DAS, ESR and CRP were decreased with the reduction of inflammation in RA and AS patients with treatment. PWV and Alx of arterial stiffness parameters decreased after 3 months of treatment, but it did not reach statistical significance. Arterial stiffness and cardiovascular risk are expected to reduce significantly with ongoing treatment process.

Keywords: Rheumatoid arthritis, ankylosing spondylitis, arterial stiffness, vascular endothelial growth factor, E-selectin, disease activity

ÖZ

Amaç: Romatoid artrit (RA) ve anklozan spondilitte (AS) endotel hasarı, damar geçirgenliğinde değişim, plak oluşumu kronik enflamasyonda rolleri olan sitokin ve kemokinlerin etkisi ile olmaktadır. Yapılan çalışmalarda enflamasyon düzeyi ile arteryel sertlik ve hücre adezyon molekül miktarında değişim olduğu bildirilmiştir. Bu çalışmada, romatizmal hastalığı olan hastalarda vasküler endotel büyüme faktörü (VEGF), E-selectin, arteriyel sertlik ve hastalık aktivitesi arasındaki ilişkiyi belirlemeyi amaçladık.

Yöntemler: Çalışmamıza AS tanılı 13 hasta, RA tanılı 28 hasta ve 30 sağlıklı kontrol dahil edildi. Tüm hasta ve sağlıklı kontrol grubunda arteriyel sertlik, VEGF ve E-selektin, eritrosit sedimentasyon hızı (ESH) ve CRP düzeylerine bakıldı. Romatizmal hastalığı bulunan grupta hastalık aktivite göstergeleri olan Banyo Ankilozan Spondilit Hastalığı Aktivite İndeksi (BASDAİ) ve DAS-28 skorları hesaplandı. Serum VEGF ve E-selektin ölçümü ELISA yöntemi ile, arteriyel sertlik ölçümü ise osilometrik metotla bakıldı. Elde edilen veriler Student t-testi, Mann-Whitney U testi ve Wilcoxon testi ile istatistiksel olarak karşılaştırıldı.

Bulgular: Tüm hasta gruplarında tedavi sonrasında 3. ayda DAS-28, BASDAİ skorları, ESH ve CRP düzeylerinde anlamlı bir azalma olduğu gözlenmiştir (p<0,001). Tedavi ile birlikte VEGF ve E-selektin düzeylerinde artış, nabız dalga hızı (NDH) ve artırma indeksi (Alx) parametrelerinde ise azalma olduğu gözlenmiştir. Non-tümör nekroz faktörü tedavisi alan RA hastalarında NDH düzeyi değişmezken, Alx düzeyi tedavinin 3. ayında azalmıştır.

Sonuç: RA ve AS hastalarında tedavi ile birlikte inflamasyonun azalması hastalık aktivite skorunda, ESH, CRP değerlerini azaltmaktadır. Arteryel sertlik parametrelerinden NDH ve Alx'i tedavinin 3. ayında azalma ile birlikte anlamlı düzeye ulaşmamıştır. Devam eden tedavi sürecinde arteryel sertliğin ve kardiyovasküler riskin anlamlı düzeyde azalacağı düşünülmektedir.

Anahtar Kelimeler: Romatoid artrit, ankilozan spondilit, arteriyel sertlik, vasküler endotelyal growth faktör, e-selektin, hastalık aktivitesi

Introduction

Rheumatic diseases such as rheumatoid arthritis (RA) and ankylosing spondylitis (AS) can cause morbidity with extra-articular involvement. Various cytokines involved in RA pathogenesis lead to synovial cell proliferation, expression of endothelial adhesion molecules and collagen production. Rapid atherosclerosis and increased risk of cardiovascular disease are also mentioned in AS and RA patients, and inflammatory processes have an important role (1-3). Atherosclerosis is more common in RA patients than in the general population (1,2). Arterial stiffness is a condition that leads to decreased expansion capacity of the vessels in response to pressure changes. Arterial stiffness is an independent risk factor for cardiovascular disease and can be used as an indicator of subclinical cardiac injury (4,5).

It is known that angiogenesis and angiogenetic factors play a role in the development of rheumatic diseases. Vascular endothelial growth factor (VEGF) is a potent angiogenic factor and oversecretion can lead to the development of many pathological conditions such as atherosclerosis, age-related macular degeneration, RA and diabetic retinopathy (6,7). E-selectin (CD62E), a cell adhesion molecule expressed in endothelial cells, plays a mediator role in the interaction between vascular endothelium and circulating leukocytes in many pathophysiological conditions such as inflammation, infection and cancer metastasis. This interaction is an important part of various pathophysiological processes such as inflammation and atherosclerosis (8).

In this study, we aimed to present the relationship between disease activation, arterial stiffness and changes in the level of cell adhesion molecules in patients diagnosed with RA and AS.

Methods

Forty-one patients (28 RA and 13 AS) (BASDAI>5 and DAS28>5.1) with rheumatic disease and 30 healthy controls were included in the study. AS was diagnosed according to the axial spondyloarthritis classification criteria and RA was diagnosed using 2010 ACR/EULAR 2010 classification criteria (9,10). Regarding healthy controls, subjects with rheumatologic or other concomitant conditions (hypertension, diabetes mellitus, chronic renal failure, liver disease, hypo-hyperthyroidism, chronic obstructive pulmonary disease, peripheral arterial disease, coronary artery disease, malignancy) were excluded from the study. Demographic and laboratory features of the patients were recorded. Informed consent form was signed by all participants. Adnan Menderes University Faculty of Medicine, Non-invasive Clinical Research Ethics Committee (decision no: 2015/739, date: 25.12.2015).

VEGF and E-selectin were measured with human ELISA kit (SunRed Biological Technology, Shanghai, China). Standard solutions of 75, 150, 300, 600, 1200 ng/L were prepared using the stock standard solution in the kit. 50 µL control, standard and samples were placed in antibody-coated wells in the plate, respectively. 50 µL chromogen/substrate solution was placed in all wells and kept at 37 °C for 10 minutes without light. ELISA (DAR 800, Diagnostic Automation, California, USA) was automatically calculated at 450 nm with the help of standard solutions prepared using a microplate reader.

Arterial stiffness was measured by using a single cuffed oscillometric "arteriograph device". Patients were rested for at least 5 minutes before measurement and were asked not to smoke or drink caffeinated beverages at least 30 minutes before. The cuff of the device was adjusted to fit in the position and at the heart level, and 3 consecutive measurements were taken automatically for 30 seconds. The augmentation index (AIx) and pulse wave velocity (PWV) levels were measured by a computer.

Statistical Analysis

The data were evaluated using SPSS version 22 statistical program. Descriptive data were expressed as mean, standard deviation, median, minimum, maximum and percentage. The obtained data were statistically compared with Student's t-test, Mann-Whitney U test and Wilcoxon test. A p value less than 0.05 was considered statistically significant.

Results

Forty-one patients and 30 healthy controls were included in this study. There were 28 patients (68.3%) diagnosed with RA and 13 patients (31.7%) diagnosed with AS. Fourteen patients (34.1%) were male and 27 (65.9%) were female. The mean age was 51.0±12.1 years (range: 27-74 years). In the control group, 10 subjects (33.3%) were male and 20 (66.7%) were female. The mean age was 51.9±10.8 (range: 29-71 years). The mean body mass index (BMI) of the patients was 30.7±6.5, while the mean BMI of the control group was 29.5±3.9. There was no statistically significant difference between patients and control groups in terms of age and BMI (p>0.05). Twenty-eight patients were using anti-tumor necrosis factor (anti-TNF) and the remaining 13 patients were using non-TNF biological agents.

A statistically significant decrease in DAS-28, BASDAI, erythrocyte sedimentation rate (ESR) and C-Reaktif Protein (CRP) levels were observed in all patients after 3 months of treatment. The pre- and post-treatment median levels of E-selectin (359.5 ng/L vs 386.5 ng/L, p=0.311) and VEGF (956.5 ng/L vs 1093.5 ng/L, p=0.046) in RA patients are shown in Table 1. The post-treatment median levels of E-selectin (414.0 ng/L vs 393.0 ng/L, p=0.724) and VEGF (1035.0 ng/L vs 1028.0 ng/L, p=0.422) were increased in the AS patients (Table 1). Despite a slight decrease in E-selectin levels and a slight increase in VEGF levels after treatment, there was no

Table 1. Serum E-selectin and vascular endothelial growth factor levels in rheumatoid arthritis and ankylosing spondylitis patients

	Rheumatoid arthritis			Ankylosing spondylitis		
	Pre-treatment (ng/L)	Post-treatment 3rd month (ng/L)	p-value	Pre-treatment (ng/L)	Post-treatment 3rd month (ng/L)	p-value
E-selectin	359.5	386.5	0.311	393.0	414.0	0.724
VEGF	956.5	1093.5	0.046	1028.0	1035.0	0.422

VEGF: vascular endothelial growth factor

Table 2. Arterial stiffness parameters in rheumatoid arthritis and ankylosing spondylitis patients

	Rheumatoid arthritis			Ankylosing spondylitis		
	Pre-treatment (ng/L)	Post-treatment 3rd month (ng/L)	p-value	Pre-treatment (ng/L)	Post-treatment 3rd month (ng/L)	p-value
AIx	28.8±9.0	26.7±10.7	0.217	27.4±8.2	27.7±10.9	0.927
PWV	8.0±1.5	7.9±1.6	0.553	6.4±0.9	6.3±0.8	0.679

AIx: augmentation index, PWV: pulse wave velocity

statistically significant difference in E-selectin and VEGF levels between anti-TNF or non-TNF treatment in both AS and RA patients. A statistically significant difference was not detected in the parameters of AIx and PWV before and after 3 months of treatment in RA and AS patients (Table 2).

Discussion

VEGF is an angiogenic cytokine that plays a role in angiogenesis, and also stimulates the migration and proliferation of endothelial cells (6). Serum and synovial fluid VEGF levels in RA patients were found to be higher than patients with osteoarthritis systemic lupus erythematosus and scleroderma, and healthy subjects (11,12). Serum VEGF levels were found to be higher in AS patients compared to healthy controls (13). In our study, we did not find any significant difference between AS and healthy control group in terms of serum VEGF level. In our study, VEGF levels were significantly lower in the patient groups than in the control group, whereas there was no statistically significant difference between AS and RA patients.

Serum VEGF levels have been reported to decrease in patients with AS treated with infliximab (14). Selaas et al. (15) found a decrease in VEGF levels on the 3rd, 6th and 12th months in RA patients treated with infliximab. In our study, VEGF levels were increased on the 3rd month of treatment, but there was no statistically significant difference compared to baseline. Positive correlation between VEGF levels, CRP and BASDAI was reported in 201 AS patients treated with infliximab (14). In our study, AS patients showed a significant decrease in BASDAI, CRP, and ESR values 3 months after treatment. On the other hand, there was no significant difference in VEGF levels compared to pre-treatment levels.

E-selectin is a cell adhesion molecule expressed on the endothelial surface in response to proinflammatory cytokines (8). E-selectin levels were investigated and no significant differences were found between the control group and AS patients (16). In a study by Baeten et al. (17), 21 patients with spondyloarthropathy were treated with anti-TNF therapy and it was reported that E-selectin levels remained unchanged in synovial examination 12 weeks after treatment. It was reported that there was no change in E-selectin and VEGF levels at the end of 14 weeks in RA patients receiving TNF-alpha (α) blockade (18). In our study, E-selectin levels did not differ significantly in both AS and RA patients compared with control group. The cause of subclinical atherosclerosis may be due to platelet parameters, which are another important component in the atherosclerotic process because AS patients have normal endothelial function (19). We also found no significant difference in E-Selectin levels between all patients with rheumatic disease and control group.

Arterial stiffness is a structural change in the arterial wall caused by increase in PWV. PWV is widely accepted as an accurate and non-invasive method for assessing arterial stiffness. Even though PWV is a direct measurement of arterial flexibility, AIx is a more complex parameter of vascular elasticity and peripheral resistance. PWV and AIx are considered independent predictors of major cardiovascular events and all-cause mortality (20). Studies have shown that arterial stiffness is increased in RA patients compared to the control group; this may contribute to increased cardiovascular risk by accelerating the atherosclerotic process (21,22). In our study, there was no significant difference in PWV and AIx parameters between RA patients and control group. Even though we observed a decrease in PWV and AIx parameters in all patient groups on the 3rd month of treatment. This decrease was not significant compared to the baseline.

We found no significant difference in the levels of AIx and PWV in RA patients treated with infliximab compared to control group. In another study comparing etanercept with methotrexate, AIx was measured on the 2nd and 4th month. It was found that AIx improved in the etanercept group and remained unchanged in the methotrexate group (23). Different results obtained from different studies suggest that these may be related to the lack of direct measurement of arterial stiffness, differences in the reflectance of peripheral in RA patients and also other poorly understood concomitant factors (22).

There are inconsistent results in the literature regarding arterial stiffness in AS patients compared with the control group. In one study, no significant difference was found in arterial stiffness between AS patients and the control group. Avram et al. (24) compared 24 AS patients with 24 controls and found that AIx and PWV levels were significantly higher in the patient group. In our study, PWV levels were significantly lower in patients with AS than in the control group. On the other hand, there was no significant difference in AIx levels between the control group and AS patient group. In AS patients, AIx value increased from 27.4±8.2 to 27.7±10.9 on the 3rd month of treatment. However, this increase was not statistically significant.

Although systemic inflammation is the primary cause of increased atherosclerosis in AS patients, the role of inflammation in stiffness of arteries is still unclear (24). When we compared AIx and PWV levels in all patient groups with the control group, we did not find any significant difference. In all patient groups, there was a significant decrease in disease activity score, sedimentation and CRP values at 3-month values after biological agent treatment. Despite the difference in AIx and PWV levels with biochemical treatment, it was not statistically significant. The main limitation of this study is that we have no information about the long-term effect of anti-TNF agents on these parameters. Another limitation is the **small sample** size.

Conclusion

In our study, there were no significant difference in the levels of E-selectin and VEGF, AIx and PWV parameters in AS patients treated with biological agents compared with pre-treatment levels. The comparison between RA and AS patients showed no significant difference in terms of VEGF, E-selectin, and also PWV and AIx. It was shown that both PWV and AIx values decreased insignificantly in RA patients who received biological treatment. PWV levels remained unchanged in RA patients receiving non-TNF-α biotherapy, but AIx levels decreased in the 3rd month of treatment. Reduction of the inflammatory process with treatment is thought to cause a decrease in the arterial stiffness, which is an important risk factor for cardiovascular diseases.

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