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Original Investigation

Relationship between asymmetric dimethylarginine and endothelial dysfunction in patients with rheumatoid arthritis

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Abstract

Objective: In rheumatoid arthritis (RA), endothelial dysfunction caused by the inflammatory process increases the risk of cardiovascular disease. Asymmetric Dimethylarginine (ADMA) leads to vascular dysfunction, whereas atherosclerosis and increased ADMA is associated with cardiovascular disease risk factors. Flow-mediated Dilation (FMD) is a radiological method to demonstrate endothelial dysfunction. In the present study, we assessed the availability of ADMA as a marker for endothelial dysfunction in RA patients. ADMA can be used as a simple and cheaper method for the determination of endothelial dysfunction.

Material and Methods: Forty patients (1 male, 39 female) diagnosed with RA according to the classification criteria and 29 healthy volunteers (2 males, 27 females) were included in this study. ADMA was studied by enzyme-linked immunosorbent assay (ELISA). Chi-square, Fisher's exact test, Mann–Whitney U test, and Spearman's correlation tests were used for analytical analysis, and $p<0.05$ was considered as the level of statistical significance.

Results: In our study, ADMA levels were significantly higher in RA patients. The ADMA level was inversely correlated with FMD. Although high levels of both C-reactive protein and ADMA were detected in patients with high disease activity, there was no statistically significant difference between these parameters ($p=0.18$). There were statistically significant negative correlations between FMD and age and disease duration ($p=0.01$, $p=0.01$). However, there were no statistically significant correlations with erythrocyte sedimentation rate, rheumatoid factor, and disease activity score ($p=0.68$). In RA patients, there was a statistically significant positive correlation between disease duration and ADMA, whereas a negative correlation was found between FMD and ADMA ($p<0.05$).

Conclusion: Our results support the hypothesis that ADMA may be used in the assessment of endothelial dysfunction in patients with RA. It will be cost-effective when commonly used. ADMA may be used in the assessment of endothelial dysfunction in patients with RA.

Keywords: Rheumatoid arthritis, endothelial dysfunction, asymmetric dimethylarginine, flow mediated dilation

Introduction

Rheumatoid arthritis (RA) is an autoimmune, chronic, inflammatory disease characterized by synovial cell proliferation, resulting in destruction of the joints and multisystem involvements (1). RA affects approximately 1% of the general population, and the average mortality risk is increased from 0.9- to 3-fold when compared with the general population, with 35%–50% of this risk being due to cardiovascular diseases (CVD) (1-3). The etiological factors for the CVD-related mortality of RA is associated with atherosclerosis as well as myocarditis, pericarditis, and valvular heart disease. It is not enough to explain an increased CVD risk with only traditional risk factors, such as smoking, hyperlipidemia, and hypertension (4, 5) because increased inflammatory activity and autoimmune factors are additional risk factors for CVD too (6).

In RA, tumor necrosis factor alpha (TNF-α), Interleukin (IL)-1, and IL-6 cause insulin resistance and dyslipidemia by affecting adipose, muscular, and hepatic tissues. They lead to vascular endothelial dysfunction, atherosclerosis, and prothrombotic events (6, 7). Cytokines increase C-reactive protein (CRP) synthesis, which is a prognostic factor for CVD associated with atherosclerosis (6-8). Also, it provides additional contributions to endothelial dysfunction and atherosclerosis by increasing the synthesis of endothelial adhesion molecules, such as intercellular adhesion molecule (ICAM)-1, vascular cell adhesion molecule (VCAM)-1, and e-selectin (6-8).

Asymmetric Dimethylarginine (ADMA) is an endogenous inhibitor for nitric oxide (NO). Increased ADMA leads to the reduction of NO synthesis, vascular dysfunction, and atherosclerosis. Increased ADMA is associated with CVD risk factors, such as dyslipidemia, smoking, renal failure, hypertension, and diabetes mellitus (9, 10). It is thought that ADMA was an important indicator for CVD in pre-clinical conditions. Flow-mediated Dilation (FMD) is a non-invasive radiological imaging method used for endothelial dysfunction de-

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termination (11). It is applicable for screening asymptomatic patients. FMD was significantly decreased in patients with CVD (12).

In our study, we aimed to determine the endothelial dysfunction by FMD and ADMA (an inhibitor of NO) in patients with RA.

Material and Methods

Forty patients (1 male, 39 female) diagnosed with RA according to the classification criteria and 29 healthy volunteers (2 males, 27 females) were included in this study. The mean age of the RA group was 43.86±7.96 years and 46.13±9.05 years for the control group. Demographic features, laboratory results [erythrocyte sedimentation rate (ESR), CRP, rheumatoid factor (RF), anti-cyclic citrullinated peptide], disease activity score (DAS-28), and physical examination of patients and healthy volunteers were recorded. Written informed consent was obtained, and the study protocol was approved by the Ethics Board of Adnan Menderes University School of Medical.

ADMA (Immundiagnostic AG; Bensheim, Germany) was studied by enzyme-linked immunosorbent assay (ELISA), and the results were automatically calculated from the ELX800 ELISA reader and data were obtained. The method described by Celermaj et al. (11) was used to measure FMD. The diameter of the brachial artery and the blood flow velocity were detected by Doppler after a 10-min rest period in a quiet environment and at a constant temperature. Later, the cuff for measurement was wrapped around the arm, and measurement was performed. It was inflated up to 250 mmHg and allowed to stand for 5 min. The artery diameter measurement was performed after 1 min. FMD and change in arterial diameter/basal artery diameter were calculated as percentages.

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software (version 17.0, SPSS Inc.; Chicago, IL, USA). The descriptive statistics were given as a number, percentage, and mean±standard deviation. Chi-square, Fisher's exact test, Mann–Whitney U test, and Spearman's correlation tests were used for analysis, and $p<0.05$ was considered as the level of statistical significance.

Results

In the present study, 40 RA patients and 29 healthy controls were evaluated. The average disease duration was 6.45±5.45 years.

The average level of ADMA was 0.55±0.20 in patients with RA and 0.29±0.08 for the control group ($p<0.05$) (Table 1). Although high levels of both CRP and ADMA were detected in patients with high disease activity, there were no statistically significant differences between these parameters ($p=0.18$).

Table 1. ADMA levels in patients with rheumatoid arthritis and in the control group

	Control group (n=29)	RA group (n=40)	p
ADMA	0.29±0.08	0.55±0.20	<0.05
ESR	8.38±5.3	31.2±17.6	
CRP	3.2±2.0	31.9±16.1	
Rf (positive, %)	0 (0%)	19 (47.5%)	
Anti-CCP (positive, %)	0 (0%)	21 (52.5%)	
DAS-28	NA	4.68±0.92	

ESR: erythrocyte sedimentation rate; CRP: C-reactive protein; Rf: rheumatoid factor; Anti-CCP: cyclic citrullinated peptide; DAS-28: disease activity score-28; NA: not analyzed; RA: rheumatoid arthritis; ADMA: asymmetric dimethylarginine

Table 2. FMD in patients with rheumatoid arthritis and in the control group

	FMD (Normal)		FMD (Decreased)		
	n	%	n	%	p
RA group	28	70	12	30	0.001
Control group	29	100	0	0	
Total	57	82.6	12	17.4	

FMD: flow-mediated dilation; RA: rheumatoid arthritis

Decreased FMD values were detected in 12 patients (30%) with RA, and all of the FMD values were measured within normal limits in the control group ($p=0.01$) (Table 2). There were statistically significant negative correlations between FMD and age and disease duration ($p=0.01$, $p=0.01$). However, there was no statistically significant correlation between sedimentation, RF, and DAS-28 ($p=0.68$).

In RA patients, there was a statistically significant positive correlation between disease duration and ADMA, whereas a negative correlation was found between FMD and ADMA ($p<0.05$).

Discussion

In our study, increased ADMA levels, decreased FMD, and therefore an increase risk for CVD were determined depending on disease duration in patients with RA. There is an increased risk for CVD and premature atherosclerosis in RA. Currently, RA is considered as an independent risk factor for CVD (13). It is believed to be caused by systemic inflammation due to increased cytokine levels, and endothelial dysfunction occurs due to vascular damage (6-8). The risk of myocardial infarction is increased 3.1-fold in RA patients who had a time from diagnosis duration of more than 10 years (14). In a study, CVD risk was reported as 13%, 5%, and 12% in nondiabetic patients with RA, the control group, and the diabetic patient group, respectively (15). Therefore, it is important to detect atherosclerosis at an early stage, to identify patients with high risk, and to initiate treatment early.

Acute coronary events are increased 3.9-fold by ADMA levels (in the highest quartile compared with the other quartiles) in middle-aged males who did not smoke (16). Studies have shown changed ADMA levels in inflammatory diseases, such as RA and ankylosing spondylitis (AS) (17-21). In a study, significantly higher ADMA levels in patients with AS were reported than in the control group (17). In another study, an inverse correlation was reported between insulin-like growth factor (IGF)-1 and ADMA levels in nondiabetic AS patients undergoing anti-TNF-αtherapy. ADMA levels were associated with features of metabolic syndrome such as hypertension (18). Di Franco et al. (19) reported significantly higher ADMA levels in patients with early-stage RA than in the control group. The ADMA levels were significantly decreased to normal range after treatment, which is an important finding and supports the association between proinflammatory mediators and NOS activity. In contrast, plasma ADMA levels did not show significant changes in patients with early RA after 18 months of treatment with methotrexate and adalimumab (20). In another study, significant positive associations were determined between plasma ADMA levels with CRP and DAS-28 but not with age in RA patients (21). The

association between ADMA and RF was reported. Seropositive patients had higher ADMA induction than seronegative patients (22). In our study, there were statistically significantly higher ADMA levels in patients with RA than in the control group, and we determined a statistically significant correlation between ADMA levels with age, disease duration, and RF. Also, we detected increased DAS-28 with increased ADMA levels. ADMA changes depend on the disease activity. However, there was no statistically significant difference between them (r=-0.04, p=0.78). ADMA is an easy method giving quick results. It will be cost-effective when it is commonly used. It may be useful to determine the cardiovascular risk of RA patients.

Increased carotid artery intima-media thickness (IMT) was reported in RA patients who were diagnosed a long time ago compared with newly diagnosed patients (23). There was a positive correlation between carotid IMT and disease duration. Also, the risk of CVD is increased by inflammatory exposure over time. FMD is a non-invasive radiological imaging method used for endothelial dysfunction determination (24). It is applicable for screening asymptomatic patients. FMD was significantly decreased in patients with CVD (25). The correlation between FMD and IMT were reported in patients with RA (7). The correlation between IMT, FMD, and disease duration was determined, and FMD measurement was proposed to evaluate CVD risk.

Significantly decreased FMD was reported in RA patients according to the study by van Doorn et al. (26). In our study, decreased FMD levels were detected in 12 patients (30%) with RA, and the levels were determined within normal limits in the control group (p=0.01). In a study by Van Doornum et al. (27), no correlation was determined between FMD and ESR, CRP, and DAS-28. These results suggest that endothelial dysfunction is not associated with instant disease activity. In our study, we also did not determine any correlations between FMD and DAS-28.

RA causes endothelial dysfunction as result of long-term inflammation and an increased CVD risk. The risk for CVD was evaluated with traditional risk factors in patients with RA. However, it has been reported that CVD risk may also be increased in patients without traditional risk factors. It is well known that tight control of systemic inflammation and clinical disease activity improves endothelial dysfunction and atherosclerosis. We believe that ADMA is a useful marker to predict CVD risk. It may be used in the assessment of endothelial dysfunction in patients with RA.

Ethics Committee Approval: Ethics committee approval was received for this study from the ethics committee of Adnan Menderes University School of Medicine.

Informed Consent: Written informed consent was obtained from patients who participated in this study.

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