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Investigation of the Correlation Between ADMA Levels and Carotid Artery Intima-Media Thickness in Rheumatoid Arthritis Patients

Romatoid Artritli Hastalarda ADMA Düzeyleri ile Karotid Arter Intima-Media Kalınlığı Arasındaki İlişkinin Saptanması

Belgin KARAOĞLAN, Gökhan Tuna ÖZTÜRK***, Vildan ÖZDOĞAN****, Ahmet CUMAOĞLU*, Suna OKTAR**, Nihal TAŞ, Aysel ARICIOĞLU*

Gazi University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Ankara, Turkey

**Gazi University Faculty of Medicine, Department of Biochemistry, Ankara, Turkey*

***Gazi University Faculty of Medicine, Department of Radiology, Ankara, Turkey*

****Nigde State Hospital, Department of Physical Medicine and Rehabilitation, Nigde, Turkey*

*****Caycuma State Hospital, Department of Physical Medicine and Rehabilitation, Zonguldak, Turkey*

Summary

Objective: Plasma asymmetric dimethylarginine (ADMA), an endogenous inhibitor of nitric oxide synthesis, is related to increased cardiovascular risks, endothelial dysfunction and atherosclerosis. Carotid artery intima-media thickness (IMT) is closely related to the risk of coronary artery disease. We aimed to investigate plasma ADMA levels and its relation to carotid IMT in patients with rheumatoid arthritis (RA).

Materials and Methods: Eighteen Turkish patients with RA (16 females, mean age: 49.44±8.88 years) and 18 age- and gender-matched healthy controls (16 females, mean age: 46.28±4.97 years) were included in the study. Measurement of IMT was done by B-mode ultrasound. Plasma ADMA levels and carotid IMT of both sides were measured in all patients and healthy controls, and the means of the two groups were compared. The correlation between ADMA levels and carotid IMT was assessed in patients with RA.

Results: Plasma ADMA levels were significantly higher in patients compared to healthy controls. Although the carotid IMT values were relatively higher in the patient group than in the control group, the difference was not statistically significant. There was no significant correlation between ADMA levels and carotid IMT values.

Conclusion: Our findings support the notion that plasma ADMA levels are elevated in patients with RA. Despite lack of correlation between ADMA levels and IMT in our study, ADMA levels can be used to evaluate endothelial dysfunction. *Turk J Phys Med Rehab 2011;57:114-8.*

Key Words: Rheumatoid arthritis, ADMA, carotid IMT

Özet

Amaç: Nitrik oksit sentezinin endojen inhibitörü olan asimetrik dimetilarginin (ADMA) plazma düzeyleri, artmış kardiyovasküler risk, endotelyal disfonksiyon ve ateroskleroz ile ilişkilidir. Karotid arter intima-media kalınlığı (IMT), koroner arter hastalık gelişimi riski ile yakından ilişkilidir. Biz bu çalışmamızda romatoid artritli hastalarda plazma ADMA düzeylerini belirlemeyi ve bunun karotid IMT ile arasındaki ilişkiyi incelemeyi amaçladık.

Gereç ve Yöntem: Araştırmamıza romatoid artrit tanısı konulan 18 Türk hasta (16 kadın, 2 erkek; yaş ortalamaları 49,44±8,88 yıl) ve 18 sağlıklı gönüllü (16 kadın, 2 erkek; yaş ortalamaları 46,28±4,97 yıl) dahil edildi. IMT ölçümü B-mode ultrasonografi ile değerlendirildi. Hasta ve sağlıklı gönüllülerin plazma ADMA düzeyleri ile her iki taraf karotid arter intima-media kalınlıkları ölçülerek iki grubun ortalamaları karşılaştırıldı. Ayrıca romatoid artritli hastaların ADMA düzeylerinin karotid arter intima-media kalınlıkları ile olan korelasyonu değerlendirildi.

Bulgular: Plazma ADMA düzeyleri hasta grubunda kontrol grubuna göre anlamlı derecede yüksekti. Karotid arter IMT değerleri ise hasta grubunda kontrol grubuna göre yüksek olmasına rağmen bu fark istatistiksel olarak anlamlı değildi. Hasta grubunda ADMA düzeyleri ile karotid IMT değerleri arasında korelasyon yoktu.

Sonuç: Bizim sonuçlarımız plazma ADMA düzeylerinin romatoid artritli hastalarda daha yüksek olduğunu göstermiştir. ADMA düzeyleri ile karotid IMT değerleri arasında korelasyon gözlenmemesine rağmen, ADMA seviyeleri endotelyal disfonksiyonun değerlendirilmesinde kullanılabilir. *Türk Fiz Tıp Rehab Derg 2011;57:114-8.*

Anahtar Kelimeler: Romatoid artrit, ADMA, karotid IMT

Address for Correspondence/Yazışma Adresi: Gökhan Tuna Öztürk MD, Niğde State Hospital, Department of Physical Medicine and Rehabilitation, Niğde, Turkey
Tel: +90 532 739 76 46 E-mail: gokhantuna06@gmail.com

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Introduction

Rheumatoid arthritis (RA) is a chronic, systemic, autoimmune, inflammatory disease affecting about 1% of the adult population and causing deterioration in quality of life (1,2). Pain, lack of power, symmetrical synovitis of diarthrodial joints, destructive changes in joints, functional losses, as well as associated cardiovascular, neurological and metabolic problems may be observed in these patients. Recent epidemiologic studies have shown that cardiovascular system problems, particularly primary coronary artery diseases, are important causes of mortality and morbidity (3,4).

The increase of cardiovascular mortality in patients with RA cannot be explained by traditional risk factors. This might be related to inflammatory activation, duration and progression of disease (5,6). Endothelial dysfunction is an early indicator of atherosclerotic disease development and is observed as a common finding in RA patients (7). This endothelial dysfunction can be improved using disease-modifying antirheumatic drugs (DMARDs) and anti-TNF agents. However, there are also RA patients with low disease activity who demonstrate endothelial dysfunction despite the long-term use of DMARDs (8,9). Recent studies have shown that bone marrow-derived endothelial progenitor cells were reduced in the peripheral blood samples of RA patients. It was found that thes cells migrated from the peripheral blood to the inflamed and hypoxic synovium or tissues and the resultant reduced circulation lead to impairment of new blood vessels (vasculogenesis) and increased cardiovascular risk through endothelial progenitor cells (10).

Recent studies have reported that the levels of plasma asymmetric dimethylarginine (ADMA) were related to increased cardiovascular risks (11,12), and that these levels were elevated in diseases like hypercholesterolemia (13), hypertriglyceridemia (14), peripheral artery diseases (15), hypertension (16), type 2 diabetes mellitus (17), and acute coronary syndrome (18,19) where the atherosclerosis rate is increased. ADMA is an endogenous inhibitor of nitric oxide (NO) synthase (20). NO synthase is impaired in patients with high ADMA levels, resulting endothelial dysfunction thereafter incresead the risk of atherosclerotic and cardiovascular disease (21).

Measurement of carotid artery intima-media thickness (IMT) by ultrasound is used for the detection of atherosclerosis during the early stage. Carotid IMT is closely related to the risk of coronary artery disease development (22).

In this study, we aimed to determine the plasma ADMA levels in patients with RA and to investigate the relation between these levels and carotid IMT.

Table 1. Baseline characteristics of study population.

	Controls (n=18)	RA patients (n=18)
No. of females, n (%)	16 (88.8)	16 (88.8)
Age, years	46.28±4.96	49.44±8.88
Disease duration, years	-	10.39±9.05
Total cholesterol, mg/dl	173.56±15.55	168.44±38.09
HDL cholesterol, mg/dl	46.78±8.14	46.16±11.86
LDL cholesterol, mg/dl	103.19±13.77	97.14±29.67
Triglycerides, mg/dl	118±40.87	125.78±55.20
Homocysteinaemia, _M/l	14.43±4.57	15.47±6.40
Creatinine, mg/dl	0.81±0.16	0.78±0.16
Glycaemia, mg/dl	90.94±10.65	92.39±13.87
CRP, mg/dl	0.53±0.78	18.46±35.48
ESR, mm/h	14.22±5.34	25.56±22.80

Materials and Methods

Selection of Patients

Our study included 18 Turkish patients (16 females, 2 males; average age: 49.44±8.88 years) who met the American College of Rheumatology (ACR) criteria for the classification of RA and 18 age- and gender-matched healthy controls (16 females, 2 males; average age: 46.28±4.97 years). This study has been started after the approval of the local ethics committee. Informed consent was obtained from patients and controls who were willing to participate. Patients with coronary artery disease and other cardiovascular problems in their history were excluded from the study. Plasma ADMA levels and carotid IMTs of both sides were measured in all patients and healthy volunteers, and the means of the two groups were compared. The correlations of ADMA levels with carotid IMT, serum erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were also evaluated in RA patients.

Evaluation of Rheumatoid Arthritis

Morning stiffness, tender and swollen joint count, disease duration, medications, and coexisting diseases were assessed. Hand and foot radiographs of each patient were analyzed for the availability of radiographic findings. Serum ESR and CRP levels were measured to evaluate the disease activity.

Laboratory Analysis

Serum ESR and CRP levels were measured by routine methods. Serum concentration of ADMA, SDMA, and arginine were determined by using a recently developed high-performance liquid chromatography (HPLC) kit (EUREKA srl-Laboratory division, Chiarvalle, Italy) with fluorescence detection. The method allows analyzing the ADMA with clean-up columns procedure and following derivatization. This method also allows analysis of arginine and SDMA. After derivatization, 50 ul of derivated plasma samples was injected in the HPLC system and analyzed by fluorescence detection. Thirty ml of H2O:acetonitrile (50:50 v/v) solution was used as mobile phase at a flow rate of 1.0 ml/min and a column temperature of 30°C. Fluorescence detection was performed at excitation and emission wavelengths of 420 and 483 nm, respectively.

Carotid Artery Ultrasound

Ultrasound imaging of the carotid arteries was carried out with the subject in the supine position. All cases were evaluated using a GE Logiq 9, B-mode ultrasound system (GE Corp. Waukesha WI) and 7.5 MHz linear array probe. IMT was defined as the distance of carotid artery from lumen-intima interface to the media-adventitia interface. IMT of the right and left main carotid arteries was measured at the sagittal plane in 1 cm proximal of the carotid bulbous dilatation. All measurements were made by manual method on the stable images obtained during US examination.

Statistical Analysis

Descriptive data were shown as mean±standard deviation (SD). Conformity of the data with normal distribution was evaluated by

the Kolmogorov-Simirnov test in both groups. Independent sample t-test was employed in both groups to compare the data observed to be in conformity with normal distribution. Since the SDs of ADMA, ESR, CRP and triglycerides were high, the Mann-Whitney U test was applied in both groups to compare these values. The Pearson's test was used to determine the correlation between plasma ADMA levels and carotid IMT; the Spearman's correlation test was used to determine the association of plasma ADMA levels with serum ESR and CRP levels in the patient group. p-value of <0.05 was accepted as statistically significant. SPSS 11.0 for Windows was used in analyzing the data.

Findings

Descriptive features of patients in both groups are shown in Table-1. Age, sex, blood lipid profile and homocysteine levels were not different among groups.

Plasma ADMA levels were meaningfully higher in the patient group (0.52±0.19 µmol) than in the control group (0.40±0.16 µmol) (Figure 1). Plasma ESR and CRP levels were significantly higher in the patient group. However, there was no correlation of ADMA levels with ESR and CRP levels in the patient group (r^1=0.14, r^2=0.12, respectively).

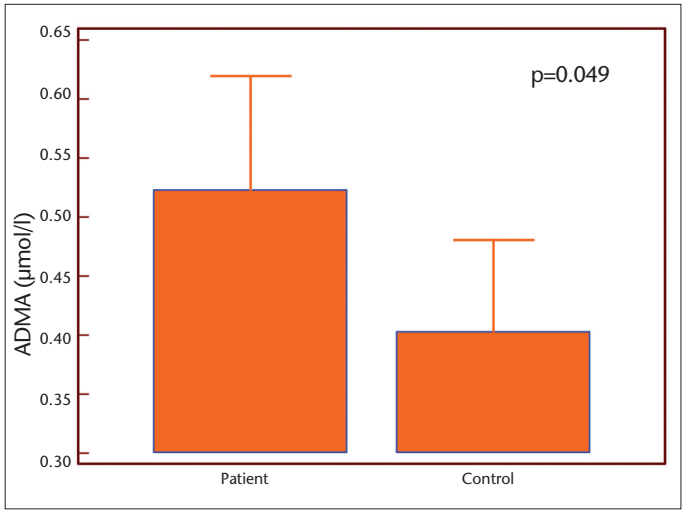

Figure 1. Plasma ADMA levels of RA patients and control group.

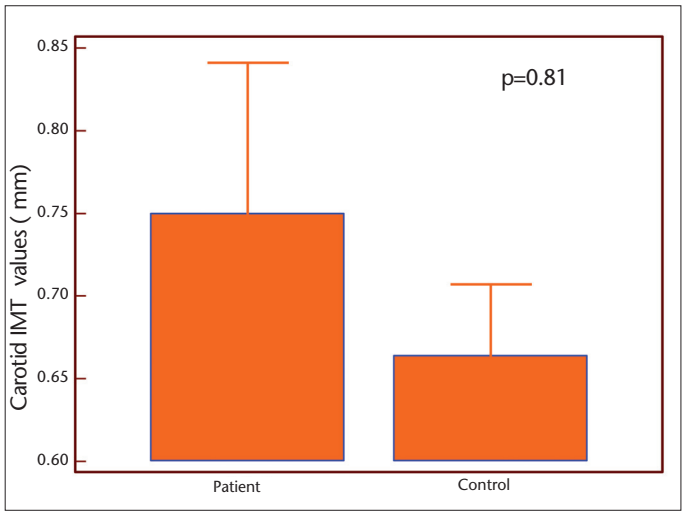

Figure 2. Carotid IMT values of patient and control groups.

Although the carotid artery IMT values were higher in the patient compared to the control group, this difference was not statistically significant (Figure 2). There was no correlation between ADMA levels and carotid IMT values in the patient group (r=0.009, p=0.97) (Figure 3). Carotid IMT values showed no correlation with the duration of disease (r=0.36, p=0.14) (Figure 4).

Discussion

Our results demonstrate that plasma ADMA levels were higher in RA patients than healthy controls. The several reasons for high ADMA levels in patients with RA can be listed as follows: 1) reduction in the activity of dimethylarginine dimethylaminohydrolase, the enzyme playing a key role in modulation of ADMA levels, 2) increase in the expression of type 1 arginine N- methyltransferase depending on the increase in oxidative stress, 3) increasing endothelial cell turnover and following protein catabolism, and 4) a decrease in dimethylarginine dimethylaminohydrolase enzyme expression in inflamed synovium due to hypoxia (23, 24). However, higher plasma ADMA levels may be seen in patients with RA depending

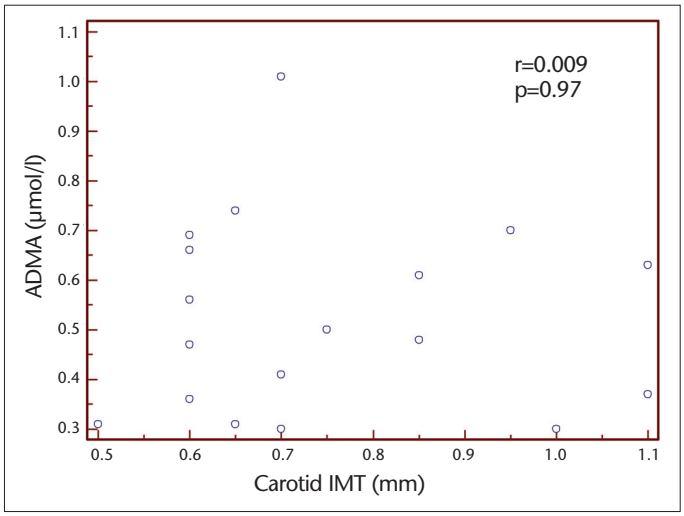

Figure 3. Correlation between plasma ADMA levels and carotid IMT values.

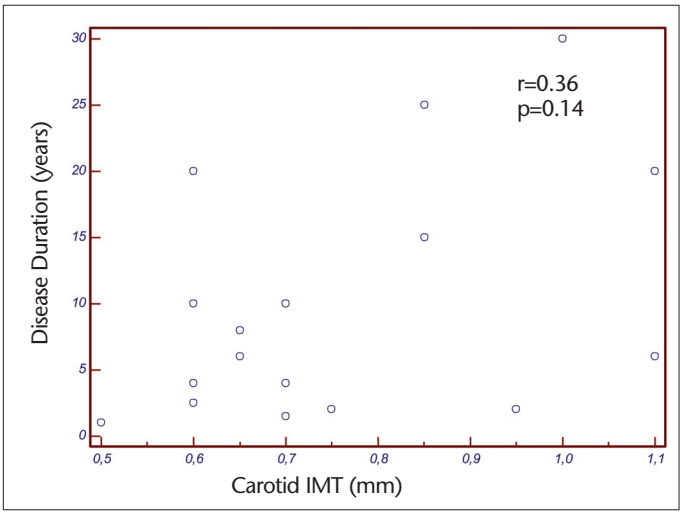

Figure 4. Correlation between carotid IMT values and disease duration.

on associated disorders. It has been previously reported by different authors that plasma ADMA levels were increased in mild renal failure (25). Stafford-Smith (26) concluded that an elevation in ADMA level as a result of decrease in kidney functions is closely related with cardiovascular disease development. However, all patients we included in this study had normal kidney functions.

Del Rincon et al. (27) reported in their study that there was an increase in carotid IMT values and atherosclerosis in patients with RA compared to healthy volunteers and this was related to the disease duration. We observed that although not statistically significantly, the carotid IMT values were higher in RA patients than in healthy controls. However, our patients' disease durations were not correlated with carotid IMT values. The advanced systemic inflammation and use of certain medication in RA may play a role in atherosclerosis development (28,29). In our study, different from the study by del Rincon et al. (29), the absence of correlation between duration of disease and carotid IMT values may be linked to the fact that several factors might play role in the atherosclerosis development.

In a study by Surdacki et al. (30), it was reported that ADMA levels and carotid IMT values were higher in patients with RA compared to the control group and that ADMA levels and carotid IMT values were correlated. Kiani et al. (31) found high ADMA levels in patients with systemic lupus erythematosus (SLE) and an association between these levels and coronary calcium level. Turiel et al. (32) observed that ADMA levels and carotid IMT values were high in patients with early RA compared to controls, and that ADMA levels and IMT values were correlated. Additionally, they reported a decreased coronary flow reserve (CFR) in patients with early RA compared to the control group and a negative correlation between carotid IMT values and CFR. However, there were no correlations between ADMA levels and carotid IMT values in our study. We thought that this would be associated with several factors playing role in the increase of plasma ADMA levels in RA. Likewise, in Turiel et al.'s (8) study investigating the effects of long-term DMARD use on endothelium dysfunction in patients with RA, it was reported that there was an increase in CFR values despite the absence of meaningful changes in ADMA and carotid IMT values after DMARD treatment. This may be explained by several factors being responsible for the increase in ADMA levels.

As a result, our findings support the argument that plasma ADMA levels are higher in patients with RA compared to healthy individuals. In patients with RA, several factors are responsible for the increase in ADMA levels and endothelial dysfunction is one of them. In our study, no correlations were seen between ADMA levels and carotid IMT values; however, ADMA levels can again be used in the evaluation of endothelial dysfunction. The small number of patients may be considered as a limitation of our study, from where we can conclude that large-population studies are needed to elucidate this subject matter.

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