Türk Biyokimya Dergisi [Turkish Journal of Biochemistry–Turk J Biochem] 2013; 38 (2) ; 169–175
doi: 10.5505/tjb.2013.51523

Research Article [Araştırma Makalesi]

Yayın tarihi 30 Haziran, 2013 © TurkJBiochem.com

[Published online 30 June, 2013]

Asymmetric dimethylarginine and arginine levels in patients with rheumatoid arthritis

[Romatoid artritli hastalarda asimetrik dimetilarjinin ve arjinin düzeyleri]

Hüsamettin Vatansev[1],
Bahadır Öztürk[1],
Sema Yılmaz[2],
Abdullah Sivrikaya[1],
Mehmet Dağlı[3],
Aysel Kıyıcı[1],
Ali Ünlü[1]

Selçuk Üniversitesi Tıp Fakültesi [1]Tıbbi Biyokimya,
[2]Dahiliye - Romatoloji Bilim Dalı
[3]Dahiliye - Hematoloji Bilim Dalı, Konya

Yazışma Adresi
[Correspondence Address]

Hüsamettin Vatansev

Selçuk Üniversitesi
Tıp Fakültesi Tıbbi
Biyokimya Anabilim Dalı
Konya
Tel. 0332 2412181
E-posta. hvatansev@selcuk.edu.tr

Registered: 5 July 2012; Accepted: 8 March 2013

[Kayıt Tarihi: 5 July 2012; Kabul Tarihi: 8 Mart 2013]

ABSTRACT

Objective: Rheumatoid arthritis (RA) is a chronic inflammatory disease and it is associated with premature atherosclerosis development. RA patients have multiple factors to develop premature atherosclerosis. Enhanced asymmetric dimethylarginine (ADMA) is considered as a risk factor for the development of atherosclerosis by inducing endothelial dysfunction. The aim of this study was to investigate the role of asymmetric dimethylarginine, arginine and citrulline levels in patients with RA and their relation to the disease activity parameters for possible role on the disease activity. **Methods:** 92 patients with RA and 34 healthy controls were included the study. Patients and control blood samples collected for ADMA, arginine and citrulline levels. ADMA, arginine and citrulline levels were measured by pre-column derivatization fluorescence HPLC method. CRP and ESR levels examined on the same day were taken from the patient records. **Results:** While ADMA levels significantly increased, arginine levels decreased in the patient group. There was no difference in citrulline levels between both groups. Only citrulline and arginine showed a weak positive correlation. ADMA, arginine, and citrulline levels showed no correlation with either biochemical parameters such as CRP or ESR, or disease activity or disease severity. **Conclusion:** Our study has shown that an increase in ADMA levels with the decrease in arginine levels suggests an increase in arginine turnover to ADMA. Increased ADMA levels can be used for an increased risk for premature development of atherosclerosis in patients with RA. Since high ADMA levels prevent the NO-dependent vasodilatation, a possible role of ADMA in development of premature atherosclerosis may be independent from inflammation.

Key Words: ADMA, arginine, citrulline, CRP, rheumatoid arthritis.

Conflict of interest: The authors report no conflicts of interest.

ÖZET

Amaç: Romatoid artrit (RA) ataklar ile seyreden kronik inflamatuar bir hastalıktır ve sıklıkla erken ateroskleroz gelişimi ile birliktedir. RA hastalarında erken ateroskleroz gelişimini hızlandıracak birçok faktör bulunmaktadır. Yüksek serum asimetrik dimetil arjinin (ADMA) düzeyleri endotel bütünlüğünü ve fonksiyonunu bozarak ateroskleroz gelişimini hızlandıran bağımsız bir faktör olarak kabul edilmektedir. Çalışmamızda RA hastalarında ADMA, arjinin ve sitrülin düzeylerini, hastalık aktivasyonundaki olası rolü içinde aktivite parametreleri ile ilişkisini araştırmayı amaçladık. **Yöntemler:** RA tanısı almış ve romatoloji kliniğinin takibinde olan 92 RA hastası ve bilinen herhangibirhastalığıolmayan34sağlıklıkontrolçalışmayadâhiledildi.SerumADMA,arjininve sitrülindüzeyleripre-kolonderivatizasyon-HPLCyöntemiilesaptandı.Rutintakipparametreleri olarak kullanılan eritrosit sedimantasyon hızı ve CRP değerleri hasta dosyasından alındı. **Bulgular:** ADMA düzeyleri RA hasta grubunda anlamlı derecede artarken, arjinin düzeylerinde azalma saptandı. Sitrülin seviyelerinde anlamlı bir değişikliğe rastlanmadı. Arjinin ve sitrülin arasında anlamlı zayıf bir korelasyon saptanırken, ADMA, arjinin ve sitrülin ile CRP, sedimantasyon hızı, hastalık şiddeti ve süresi arasında önemli bir ilişki saptanmadı. **Sonuç:** Bulgularımızda yüksek ADMA düzeylerinin azalan arjinin düzeyleri ile birlikte olmasının romatoid artritte artan arjinin-ADMA dönüşümünün bir göstergesi olabileceği yanında, bu hastalarda artan prematür ateroskleroz gelişiminde de rol oynayabileceğini göstermektedir. Yüksek ADMA düzeylerinin NO bağımlı vazodilatasyonu engellemesi nedeni ile prematür ateroskleroz gelişiminde, ADMA'nın rolünün inflamasyondan bağımsız olabileceğini düşündürmektedir.

Anahtar Kelimeler: ADMA, arjinin, sitrülin, CRP, romatoid artrit.

Çıkar Çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory disease of the joints and extra-articular tissues. Patients with RA have reduced life expectancy when compared with the general population, due to mainly increased cardiovascular events [1]. RA is characterized with accelerated endothelial dysfunction and consequently increased premature atherosclerosis [2]. The presence of subclinical coronary atherosclerosis has been demonstrated in RA patients [3]. Although the reason for increased atherosclerosis can be explained by traditional factors, the development of atherosclerosis in some of the RA patients still cannot be fully explained by these factors [4]. Therefore, several studies have projected to search some other factors such as adhesion molecules, C-reactive protein (CRP), oxidative stress parameters and endothelin levels [1,2,4,5].

One of the main reasons and consequence of endothelial dysfunction is impaired nitric oxide (NO) bioavailability [6]. NO bioavailability is mostly regulated by the activity of nitric oxide synthase (NOS, EC.1.14.13.39). NOS can be competed by substrates, endogenous L-arginine and asymmetric dimethylarginine (ADMA). ADMA, as a competitive endogenous inhibitor of NOS, has an important role in the synthesis of NO [7]. ADMA is a product of the catabolism of proteins containing methylated arginine residues [8, 9] and it is metabolized to citrulline by dimethylarginine dimethylaminohydrolase (DDAH, EC.3.5.3.18) [10]. Besides inhibiting NO synthesis, ADMA can directly induce oxidative stress and cell apoptosis, and participate in the inflammation reactions [11, 12].

Increased atherosclerosis development has been shown in rheumatoid arthritis (RA) patients when compared to healthy controls. In order to find the increased risk of atherosclerosis, new factors have been investigated in addition to classical factors. The aim of this study is to investigate the role of asymmetric dimethylarginine, arginine and citrulline levels in the patients with RA and their relation to the disease activity parameters.

Materials and methods

Study Population

The subjects enrolled in our study were recruited from 92 RA patients (13 male) and 34 healthy controls (19 male). RA was defined on the basis of The American College of Rheumatology (ACR) criteria using self-reports of morning stiffness and objective findings of synovitis and rheumatoid factor (RF) positivity [13]. Radiographs of the hands and wrists were obtained for all of the subjects. The clinical assessment included the number of tender and swollen joints, the duration of morning stiffness and erythrocyte sedimentation rate (ESR) and CRP levels. Disease severity was assessed using the disease activity score (DAS28) criteria [14]. Clinical assessments of the patients were done by rheumatology physician in rheumatology clinic. Exclusion criteria were as follow: The use of medications for hypertension, cardiovascular diseases, diabetes mellitus, renal and hepatic diseases, and active infectious diseases. All serum samples were stored at -80°C for ADMA, arginine and citrulline analysis. The protocol was approved by the Ethics Committee of the Selcuklu Medical Faculty (Protocol No:24.06.2010-2010/05) and all subjects volunteered for the trial and written consent was obtained according to the Declaration of Helsinki.

ADMA, Citrulline and Arginine Analysis

Measurements of ADMA, arginine and citrulline levels were accomplished by high performance liquid chromatography (HPLC), using the method described by Chen *et al* [15] with minor modifications. Shorter run time (35 to 26 minutes) and different gradient program were used as modifications. In brief, 20 mg 5-sulfosalisilic acid was added to 1 ml serum, and the mixture was left in an ice bath for 10 min. The precipitated protein was removed by centrifugation at 2,000×g for 10 min. 10 µl of the supernatant, which was filtered through a 0.45-µm pore size filter, was mixed with 100 µl derivatization reagent [prepared by dissolving 10 mg *o*-phtaldialdehyde in 0.5 ml methanol, 2 ml 0.4 m borate buffer (pH 10.0), and 30 µl 2-mercaptoethanol] and then injected into the chromatographic system (HP Agilent 1100, Agilent Technologies, Palo Alto, CA, USA). Separation of ADMA, arginine and citrulline was achieved with a 150×4.6 mm interior diameter Thermo ODS column with a particle size of 5 µm (Thermo, PA, USA) using 50 mm sodium acetate (pH 6.8), methanol, and tetrahydrofurane as the mobile phase (A, 82:17:1; B, 22:77:1) at a flow rate of 1.0 ml/min. Gradient program was used for the separation of other amino acid peaks from ADMA, arginine and citrulline peaks. Gradient program was 79% A, 21% B for 0-3.96 minutes, 75.7% A, 24.3% B for 3.96-10.56 minutes, 58.6% A, 41.4% B for 10.56-18.48 minutes, 37.2% A, 62.8% B for 18.48-21.12 minutes, 22.2% A, 77.8% B for 21.12-23.10 minutes, 79.9% A, 20.1% B for 23.10-26.00 minutes. The areas of peaks detected by fluorescent detector (excitation, 338 nm; emission, 425 nm) were used for quantification. As shown in graph 1, citrulline, arginine and ADMA standard and samples peaks were eluted at 11.30, 12.28, 12.71 minutes respectively in the same run. Linearity was assessed in the range 0.1–20 μM of ADMA. The mean correlation coefficient was >0.98. The ADMA limit of quantitation (LOQ) was 0.1 μM. Analytical recovery was 96.5 %, and the interassay coefficient of variation was less than 5%.

The ESR and CRP levels were obtained from the patients records. At the same time the obtained samples were used to find any correlations between ADMA, arginine, citrulline and the inflammatory markers.

Statistical Analysis

Data were analyzed by using statistical software and presented as mean ± standard deviation (SD) for normally distributed variables, median (min-max) for non-parametric values. The distribution of the variables was analyzed with the Shapiro–Wilk test. Independent Student's t-tests were used for comparing differences of normally distributed variables between two groups. Mann Whitney U test were used for non-parametric distributed values. Correlation analysis was carried out with Pearson's and Spearman's correlation test. Correlation coefficient "r" was accepted as no and weak correlation, moderate and strong correlation <0.25, 0.25-0.5, >0.50 respectively [16]. The study parameters were compared with disease activitiy score (DAS28) using analysis of variance Kruskal-Wallis Test. "p" value of <0.05 was considered statistically significant for all the tests.

Results

Demographic features of healthy controls and patients with RA were given in Table 1. There were no significant differences between the groups in terms of the age, BMI, blood pressure and smoking status. Because of ADMA correlates with high blood pressure, arterial blood pressures were measured for all study participants for optimizing patients and control populations. Since RA disease is more frequent in women than men, our study population has also higher number of women with RA patient. Onat *et al.* showed that ADMA levels were similar between in middle age Turkish women and men [17]. Our results also show similar ADMA, arginine and citrulline levels between the genders (data not shown). Median disease duration was 48 months (min-max:2-360). 15 RA patients were scored as high disease activity (DAS28 >5.1), 45 RA patients were scored as low disease activity (DAS28 <3.2) and 32 patients were classified as remission (DAS28 <2.6). The mean ESR of patients was nearly two fold of the controls. Median CRP levels were also significantly higher in the patients group when compared to control group (Table-1).

Biochemical parameters of healthy controls and patients with RA were given in Table 2. ADMA levels were found to have increased in patients with RA when compared to controls (p: 0.011). In contrast to ADMA levels, arginine levels were significantly decreased in patients with RA (p: 0.037). Arginine/ADMA ratio was found to be less than control in the patient group (p: 0.001). Citrulline levels were similar in the two groups.

When patients were divided according to the disease severity, no difference was observed for ADMA levels (Table 3). Similarly arginine and citrulline levels did not differ between high, low disease activity and remissi-

Table 1. Demographic features of healthy controls and patients with rheumatoid arthritis.

	Controls (n=34)	RA (n=92)	p
Age	46.76 ± 11.83	43.76 ± 12.81	NS
BMI (kg/m^2)	27.93 ± 4.83	27.28 ± 5.45	NS
Male/Female	19/15	13/79	0.01
Smoking (+/-)	7/27	12/80	NS
Arterial blood pressure (Systolic / Diastolic) (mmHg)	118.9 ± 25.83 / 77 ± 12.8	122.3 ± 21.4 / 82 ± 16.5	NS
Disease duration (month)	-	48 (2-360)	
ESR (mm/h)	9 (3-23)	21 (2 – 75)	0.001
CRP (mg/L)	5.3 (2.9-18)	27.3 (2.9-175)	0.001

Values are expressed as mean ± standard deviation. Disease duration, CRP and ESR values were given as median (min-max). *BMI:* body mass index, *NS:* not significant, *RA:* rheumatoid arthritis, *ESR:* erythrocyte sedimentation rate, *CRP:* C reactive protein .

Table 2. ADMA, arginine, arginine/ADMA ratio and citrulline levels of patients with RA and control group.

	Controls (n=34)	RA (n=92)	p
ADMA (µmol/L)	3.24 ± 1.44	4.6 ± 2.64	0.011
Arginine (µmol/L)	131.04 ± 42.78	117.99 ± 29.12	0.037
Arginine/ADMA	47.03 ± 21.35	35.93 ± 26.87	0.001
Citrulline (µmol/L)	94.27 ± 21.94	89.01 ± 18.58	NS

Values are expressed as mean ± standard deviation. *RA:* rheumatoid arthritis, *ADMA:* Asymmetric dimethyl arginine, *NS:* not significant.

Table 3. Analysis of variance of the study parameters in patients with RA divided into 3 groups according to the DAS28 score.

	High disease activity (DAS28 >5.1) (n=15)	Low disease activity (DAS28 <3.2) (n=45)	Remisssion (DAS28 <2.6) (n=32)	p
ADMA (µmol/L)	5.01 ± 2.79	4.23 ± 2.01	3.98 ± 2.25	0.51
Arginine (µmol/L)	120.98 ± 29.02	116.27 ± 24.59	125.29 ± 34.15	0.44
Arginine/ADMA	35.84 ± 26.40	35.97 ± 23.10	39.83 ± 22.20	0.30
Citrulline (µmol/L)	87.19 ± 15.88	86.55 ± 19.42	88.86 ± 18.26	0.73
ESR (mm/h)	32 (7-63)	17,5 (2-47)	12 (2-75)	0.013
CRP (mg/L)	13.70 (2.97-147)	8.30 (2.9-175)	3.97 (2.9-23.8)	0.022

Values are expressed as mean ± standard deviation. CRP and ESR values were given as median (min-max). *RA:* rheumatoid arthritis, *ADMA:* Asymmetric dimethyl arginine, *ESR:* erythrocyte sedimentation rate, *CRP:* C reactive protein.

Table 4. Correlation analysis of ADMA, arginine, citrulline, arginine/ADMA ratio, ESR, CRP DAS 28 score and disease duration.

	ADMA	Arginine	Arginine / ADMA	Citrulline	ESR	CRP	DAS28 Score	Disease Duration
ADMA	-	r: -0.12	r: -0.62**	r: 0.02	r: 0.04	r: - 0.11	r.0.1	r: 0.02
Arginine	r: -0.12	-	r: 0.47*	r: 0.35*	r: -0.09	r: 0.08	r:0.02	r: -0.08
Arginine /ADMA	r: -0.62**	r: 0.47*	-	r: 0.10	r: 0.10	r: 0.05	r:0.01	r: -0.05
Citrulline	r: 0.02	r: 0.35*	r: 0.10	-	r: -0.17	r: -0.08	r:-0.01	r: -0.03
ESR	r: 0.04	r: -0.09	r: 0.10	r: -0.17	-	r: 0.26*	r:0.35**	r: 0.13
CRP	r: -0.11	r: 0.08	r: 0.05	r: -0.08	r: 0.26*	-	r:0.38**	r: 0.38**
DAS28 Score	r.0.1	r:0.02	r:0.01	r:-0.01	r:0.35**	r:0.38**	-	r:0.23
Disease Duration	r: 0.02	r: -0.08	r: -0.05	r: -0.03	r: 0.13	r: 0.38**	r:0.23	-

* $p< 0.05$, ** $p< 0.01$.

Abbreviations: *ADMA* Asymmetric dimethyl arginine, *ESR* erythrocyte sedimentation rate, *CRP* C reactive protein.

on groups of disease. CRP and ESR levels were significantly increased in high disease activity group since they are a part of DAS 28 calculation.

Correlation studies showed significant negative correlations between ADMA and arginine /ADMA ratio (Table 4). Arginine levels correlated with arginine/ADMA ratio and citrulline levels. Arginine and citrulline levels were also showed a slight positive correlation (r:0,35, $p<0.05$). CRP levels were also positively correlated with ESR (r: 0.26, $p<0.05$) and disease duration (r: 0.38, p: 0.001). CRP and ESR levels correlated with the disease severity.

59 RA patients were diagnosed and taken into follow up program for more than 24 months. When the patients were divided into two groups according to their disease duration, 59 patients had the disease longer than 24 months. There were no differences in the parameters between diseases duration longer or less than 24 months (Figure 2).

Discussion

RA is associated with enhanced premature atherosclerosis and early impaired endothelial function after the onset of the disease [1, 2, 4]. Cardiovascular disease is one of the leading causes of morbidity and mortality and it represents about 40% of the mortality in RA patient [18]. Increased inflammatory activity, use of corticosteroids and methotrexate contribute to the development of atherosclerosis. Although inflammatory activity is important for the development of atherosclerosis [19], ethiopathological mechanisms are still unclear for the premature development of atherosclerosis and its-relation with morbidity in RA patients.

Our results demonstrated that ADMA levels were significantly higher in RA patients when compared to controls. None of our patients had high blood pressure or clinical evidence of any cardiovascular or renal disease. Sandoo *et al.* demonstrated that ADMA levels

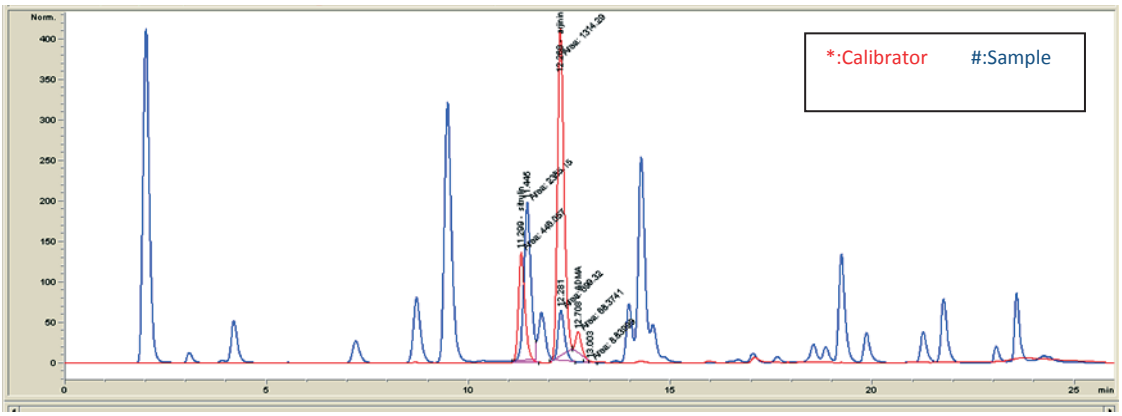

Figure 1. ADMA, arginine and citrulline standards and a representative chromatogram of patients' sample.

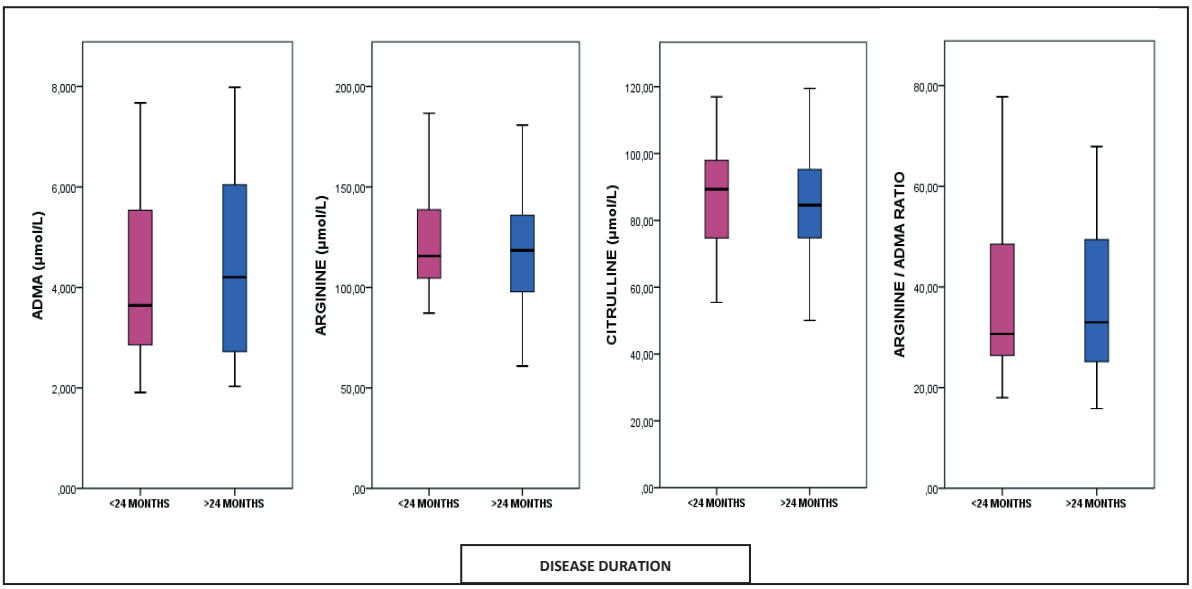

Figure 2. Study parameters levels with respect to disease duration.

increased in patients with RA but there was no significant correlation with *in vivo* assessments of endothelial function [20]. Coronary artery abnormalities have been shown in RA patients who have no clinical evidence of heart disease [3]. Reduced coronary flow reserve (CFR) has also been negatively correlated with ADMA levels in the early RA patients. Therefore, the authors suggested that increased ADMA levels were related with the decrease in CFR [3]. Although one study [21] showed an association between ADMA and common carotid intimae media thickness (IMT), other studies failed to find a relation between ADMA levels and IMT [22, 23]. A decrease in DDAH activity may be one of the reasons of high ADMA levels in the RA patients. In addition to that the decrease in arginine levels may also suggest an increase in the arginine N-methyl transferase (EC.2.1.1.2) activity in the patients. In normal situation a small fraction of arginine, about 1.5%, enters to the ADMA synthesis [24]. Decreased arginine and increased ADMA levels may also suggest that an increase in turnover of arginine to ADMA occurs in patients with RA. Since arginine is in the center of many metabolic pathways, it is difficult to conclude whether decrease in arginine levels is a consequence or cause of the disease. However both decrease in arginine and increase in ADMA levels suggest that NO synthesis impaired in RA patients. Although none of the patients had any signs of cardiovascular disease, low arginine/ADMA ratios may suggest a role to ADMA as an early indicator for the development of premature atherosclerosis. Surdacki *et al.* suggested that increased serum ADMA levels may lead to increased vascular resistance and elevated blood

pressure via decrease of NO bioavailability [25]. Intravenously administered suppressor doses of ADMA has increased arterial stiffness, decreased cerebral blood flow in young healthy men [26] and increased systemic vascular resistance, and mean arterial blood pressure in healthy subjects. In addition to that ADMA infusion has decreased cardiac output by 15% [27].

The relation of ADMA and inflammation is still in debate in the literature. ADMA has been suspected for the development of inflammatory response and proposed that ADMA may be a link between inflammation and endothelial dysfunction in humans. Antoniades *et al.* also suggested that systemic, low-grade inflammation leads to increased ADMA [28]. A strong positive correlation between CRP, fibrinogen and plasma ADMA levels has been shown in a study [29]. In our study we did not find any correlation between ESR and ADMA, arginine or citrulline. Furthermore, ADMA levels correlated with neither disease duration nor disease severity. In addition to that, CRP levels did not correlate with study parameters either. CRP levels correlated with ESR and disease duration. Our results support both Turiel *et al.* [22, 30] and Surdacki *et al.* studies [21, 31] in terms of the relation between ADMA and inflammation. Surdacki *et al.* displayed that neither ADMA nor SDMA (symmetric dimethylarginine) were correlated with the disease activity and inflammatory activation [21, 31]. It has also been shown [30] that anti-inflammatuar therapy had no effect on ADMA levels. Methotrexate and anti-tumor necrosis factor-α agents treatment of patients with RA reversed the disease activity score, decreased CRP levels and improved CFR. However, the use of methotrexate and anti-tumor necrosis factor-α agents did not change the ADMA levels and IMT measurements. Therefore, the authors suggested that improvements were independent form ADMA and IMT [30]. Our data also showed ADMA levels and arginine/ADMA ratios were similar between the disease severity forms as classified by DAS 28 score (Table 3). Since DAS 28 score is calculated mainly from inflammatory markers either laboratory or clinical signs and it shows the current patients situations, we can conclude that ADMA levels have no direct relation with the inflammatory activities in RA patients. Conflict results in the literature are probably based on the complex effect of nitric oxide in inflammation. However, the well-known fact is that inflammation is accompanied by vasodilatation of the vessels. Unavailable NO, because of high ADMA, results with enhancement vasoconstriction of the vessels. Increased vasoconstriction can also prevent inflammation.

On the other hand, a recent meta-analysis concluded that oral L-arginine supplementation is effective in improving vascular endothelial function [32]. In our study, we also find low arginine levels in RA patients. Since arginine metabolism is very complex, and only small amount of arginine enters the NOS pathway [24]. It is difficult to find a direct relation with low arginine levels and accelerated atherosclerosis. Since ADMA is a methylated derivative of arginine, it can be said that increased ADMA turnover is one of the reasons for decreased arginine levels.

Citrulline supplementation prevented the development of pulmonary hypertension and increased NO production in piglets exposed to chronic hypoxia [33]. NO, urea formation and catabolism of ADMA result with citrulline synthesis. Ananthakrishnan M *et al.* [33] suggest that neonates exposed to prolonged periods of hypoxia may potentially benefit from citrulline supplementation. In addition to that, citrulline supplementation improved arterial stiffness in middle-aged men [34]. Although above studies suggest some beneficial effects of citrulline on to vascular endothelial, it is difficult to interpret the direct effect of the serum citrulline and arginine levels on endothelial function. The complex regulation of NO synthesis and intracellular availability of arginine like its precursor and citrulline make more complicated NO bioavailability. According to the data it is difficult to conclude whether citrulline supplementation is beneficial for RA patients.

ADMA could be a useful parameter for follow-up the development of cardiovascular disease in RA patient, especially when traditional screening tests are normal. Our results suggest that RA patients with high ADMA levels should be screened carefully for the development of cardiovascular disease.

Difficulties in the classification of the patients according to the use of medication are the limitation of this study. Varieties of the disease drug, different dosage use and time made impossible for statistical analysis in some situations.

Conclusion

Our data emphasize that ADMA plays an independent role from inflammation to development of atherosclerosis in patients with RA. In our study, we revealed that ADMA levels could be used for an increased risk for premature development of cardiovascular disease but not for inflammation in RA patients. Lowering ADMA strategies may be one of the therapeutic approaches to prevent premature atherosclerosis development in RA patients. Further case-control studies especially with larger participants are required to confirm our results.

Conflict of interest: The authors report no conflicts of interest.

References

[1] AE.Voskuyl. The heart and cardiovascular manifestations in rheumatoid arthritis. Rheumatology 2006;45:iv4-7.

[2] Bisoendial RJ, Stroes ES, Tak PP. Critical determinants of cardiovascular risk in rheumatoid arthritis. Curr Pharm Des 2011;17(1):21-26.

[3] Atzeni F, Sarzi-Puttini P, De Blasio G, Delfino L, Tomasoni L, Turiel M. Preclinical impairment of coronary flow reserve in patients with rheumatoid arthritis. Ann N Y Acad Sci 2007;1108:392-97.

[4] Shoenfeld Y, Gerli R, Doria A, Matsuura E, Cerinic MM, *et al*. Accelerated atherosclerosis in autoimmune rheumatic diseases. Circulation 2005;112(21):3337-47.

[5] Firestein GS, Edward D Harris JR. Rheumatoid Arthritis. In (Ed. Ruddy S, Harris ED, Sledge CB) Kelley's Textbook of Rheumatology 2005; pp.996-1073, 6th Edition. Philadelphia, WB Saunders.

[6] Erbil MK, Kurt YG, Yaman H, Çakır E, Akgül EÖ, Çaycı T. Metabolism of asymmetric dimethylarginine and its clinical significance Turk J Biochem 2012;37 (1):99–105.

[7] Rosselli M, Keller PJ, Dubey RK. Role of nitric oxide in the biology, physiology and pathophysiology of reproduction. Human Reproduction Update 1998;4(1):3–24.

[8] Ueda S, Kato S, Matsuoka H, Kimoto M, Okuda S, *et al*. Regulation of cytokine- induced nitric oxide synthesis by asymmetric dimethylarginine: role of dimethylarginine dimethylaminohydrolase. Circ Res 2003;92(2):226-33.

[9] Zakrzewicz D, Eickelberg O. From arginine methylation to ADMA: a novel mechanism with therapeutic potential in chronic lung diseases. BMC Pulm Med 2009; 9(5):1-7.

[10] Fiedler L. The DDAH/ADMA pathway is a critical regulator of NO signalling in vascular homeostasis. Cell Adh Migr 2008;2:149–150.

[11] Wells SM, Holian A. Asymmetric dimethylarginine induces oxidative and nitrosative stress in murine lung epithelial cells. Am J Respir Cell Mol Biol 2007;36:520–28.

[12] Yuan Q, Jiang DJ, Chen QQ, Wang S, Xin HY, *et al*. Role of asymmetric dimethylarginine in homocysteine-induced apoptosis of vascular smooth muscle cells. Biochem Biophys Res Commun 2007;356:880–85.

[13] Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, *et al*. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.

[14] Cush JJ, Weinblatt ME, Kavanaugh A. Rheumatoid Arthritis: Early Diagnosis and Treatment. 2010; pp.319-20, 3rd Edition, Professional Communications, New York.

[15] Chen BM, Xia LW, Zhao RQ. Determination of N^G, N^G dimethylarginine in human plasma by high-performance liquid chromatography. J Chromatography B: Biomedical Seciences And Applications 1997;692:467–71.

[16] Saunders BD, Trapp RG. Basic & Clinical Biostatistics 1994:pp.53-54, 2nd Edition, Silver Arch Books Press, St Louis MO U.S.A.

[17] Onat A, Hergenç G, Can G, Karabulut A. Serum asymmetric dimethylarginine levels among Turks: association with metabolic syndrome in women and tendency to decrease in smokers. Arch Turk Soc Cardiol 2008;36(1):7-13.

[18] Gerli R, Goodson NJ. Cardiovascular involvement in rheumatoid arthritis. Lupus 2005;14:679-82.

[19] Hochberg MC, Silman AJ, Smolen JS, Weinblatt ME, Weisman MH. Rheumatology. 2003; pp. 753-807, 3rd Edition. Mosby, New York.

[20] Sandoo A, Dimitroulas T, Veldhuijzen van Zanten JJ, Smith JP, Metsios GS, *et al*. Lack of association between asymmetric dimethylarginine and in vivo microvascular and macrovascular endothelial function in patients with rheumatoid arthritis. Clin Exp Rheumatol. Clin Exp Rheumatol 2012;30(3):388-96.

[21] Surdacki A, Martens-Lobenhoffer J, Wloch A, Marewicz E, Rakowski T, *et al*. Elevated plasma asymmetric dimethyl-L arginine level are linked to endothelial progenitor cell depletion and carotid atherosclerosis in rheumatoid arthritis. Arthritis & Rheumatism 2007; 56(3):809-19.

[22] Turiel M, Atzeni F, Tomasoni L, de Portu S, Delfino L *et al*. Non-invasive assessment of coronary flow reserve and ADMA levels: a case-control study of early rheumatoid arthritis patients. Rheumatology (Oxford) 2009;48(7):834-39.

[23] Karaoğlan B, Öztürk GT, Özdoğan V, Cumaoğlu A, Oktar S, *et al*. Investigation of the Correlation Between ADMA Levels and Carotid Artery Intima-Media Thickness in Rheumatoid Arthritis Patients. Turk J Phys Med Rehab 2011;57:114-18.

[24] Luiking YC, Ten Have GA, Wolfe RR, Deutz NE. Arginine de novo and Nitric oxide production in disease states. Am J Physiol Endocrinol Metab 2012;303(10):E1177-89

[25] Surdacki A, Nowicki M, Sandmann J, Tsikas D, Boeger RH, *et al*. Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. J Cardiovasc Pharmacol 1999;33(4):652-8.

[26] Kielstein JT, Donnerstag F, Gasper S, Menne J, Kielstein A, *et al*. ADMA increases arterial stiffness and decreases cerebral blood flow in humans. Stroke 2006;37(8):2024-29.

[27] Achan V, Broadhead M, Malaki M, Whitley G, Leiper J, *et al*. Asymmetric dimethylarginine causes hypertension and cardiac dysfunction in humans and is actively metabolized by dimethylarginine dimethylaminohydrolase. Arterioscler Thromb Vasc Biol 2003; 23(8):1455-59.

[28] Antoniades C, Demosthenous M, Tousoulis D, Antonopoulos AS, Vlachopoulos C, *et al*. Role of asymmetrical dimethylarginine in inflammation-induced endothelial dysfunction in human atherosclerosis. Hypertension 2011; 58(1):93-98.

[29] Kwasny-Krochin B. Gluszko P. Undas A. Plasma asymmetric dimethyl-L-arginine (ADMA) in active rheumatoid arthritis: links with oxidative stress and inflammation. Pol Arch Med Wewn 2012; 122, 270–76.

[30] Turiel M, Tomasoni L, Sitia S, Cicala S, Gianturco L, *et al*. Effects of long-term disease-modifying antirheumatic drugs on endothelial function in patients with early rheumatoid arthritis. Cardiovasc Ther 2010;28(5):e53-64.

[31] Surdacki A, Martens-Lobenhoffer J, Wloch A, Gluszko P, *et al*. Plasma asymmetric dimethylarginine is related to anticitrullinated protein antibodies in rheumatoid arthritis of short duration. Metabolism 2009; 58(3):316-18.

[32] Bai Y, Sun L, Yang T, Sun K, Chen J, *et al*. Increase in fasting vascular endothelial function after short-term oral L-arginine is effective when baseline flow-mediated dilation is low: a meta-analysis of randomized controlled trials. Am J Clin Nutr 2009; 89: 77-84.

[33] Ananthakrishnan M, Barr FE, Summar ML, Smith HA, Kaplowitz M, *et al*. L-Citrulline ameliorates chronic hypoxia-induced pulmonary hypertension in newborn piglets. Am J Physiol Lung Cell Mol Physiol 2009; 297: L506-11,

[34] Ochiai M, Hayashi T, Morita M, Ina K, Maeda M, *et al*. Short-term effects of l-citrulline supplementation on arterial stiffness in middle-aged men. Int J Cardiol. 2012;155(2):257-61.