

The impact of type 2 diabetes duration on serum asymmetric dimethylarginine and C-reactive protein concentration in Bosnian patients

Asija ZACIRAGIC, Amela DERVISEVIC, Nesina AVDAGIC, Nermina BABIC, Amina VALJEVAC, Orhan LEPARA, Jasminko HUSKIC

Department of Human Physiology, Faculty of Medicine, University of Sarajevo, Sarajevo, Bosnia and Herzegovina
E-mail: asija.zaciragic@mf.unsa.ba

Objective. The present study assessed the impact of type 2 diabetes mellitus (T2DM) duration on the serum asymmetric dimethylarginine (ADMA) and C-reactive protein (CRP) concentration in Bosnian patients.

Methods. Participants for this cross-sectional study were randomly selected from the Family Medicine Clinic (Sarajevo, Bosnia and Herzegovina). Serum ADMA concentration was determined by ELISA. Serum high-sensitivity (hs-CRP) was determined by particle-enhanced immunonephelometry. ANOVA test followed by Scheffe post-hoc test or Kruskal-Wallis test followed by Man-Whitney test were used for statistical analysis.

Results. The study included 38 patients in up to 10 years diabetes duration (≤ 10 years T2DM) group, 22 patients in greater than 10 years diabetes duration (> 10 years T2DM) group, and 60 controls. Serum ADMA concentration in the > 10 years T2DM group (1.81 ± 0.15 $\mu\text{mol/L}$) was significantly higher compared to serum ADMA concentration in the ≤ 10 years T2DM group (1.38 ± 0.41 $\mu\text{mol/L}$; $p < 0.001$) and in controls (0.62 ± 0.15 $\mu\text{mol/L}$; $p < 0.001$). A significant difference in serum ADMA concentration was found between the < 10 years T2DM group and the controls ($p < 0.001$). The serum CRP concentration in the > 10 years T2DM group [5.95 (4.20 – 9.12) mg/L] was significantly higher compared to serum CRP concentration in the < 10 years T2DM group [2.35 (1.40 – 4.30) mg/L ; $p < 0.001$] and controls [0.85 (0.50 – 1.30) mg/L ; $p < 0.001$]. Significant difference in serum CRP concentration was observed between the < 10 years T2DM group and controls ($p < 0.001$).

Conclusions. The present study showed an increase in the serum ADMA and CRP concentrations with the advancement of T2DM. These results suggest that ADMA and CRP may serve as indicators of endothelial dysfunction and chronic low-grade inflammation progression in patients with T2DM. Larger prospective studies are required to confirm the observed findings.

Key words: asymmetric dimethylarginine, C-reactive protein, endothelial dysfunction, systemic low-grade inflammation, type 2 diabetes mellitus

One of the key features of diabetes is an impaired endothelium-dependent vasodilatation due to decreased bioavailability of nitric oxide (NO), a major vasodilator in humans. The endothelial dysfunction (ED) seen in diabetes is thought to be an outcome

of insulin resistance, hyperglycemia, and chronic systemic low-grade inflammation. In diabetes, micro-vascular complications are characterized by increased production of inflammatory factors, enhanced oxidative stress, impaired endothelial repair, and

abnormal angiogenesis along with a diminished release of NO (Shi and Vanhoutte 2017). Cardiometabolic risk factors such as obesity, hypertension, and dyslipidemia also significantly contribute to ED in the diabetic patients (Rajendran et al. 2013). Studies have shown that insulin has vasodilator properties through induction of NO production in the micro-circulation (Baron and Brechtel 1993). Hence, a lack or resistance to insulin leads to the NO formation reduction and a consequent ED.

One of the most important diabetes-related concerns in people with diabetes type 1 and type 2 are long-term complications. It has been proposed by numerous authors that the long-term complications mostly develop in diabetic patients after 10 years of diabetes duration (Kuniss et al. 2019). The quality report of North Rhine Westphalia, which includes clinical and laboratory data of patients enrolled in the disease management program published in 2016, has shown that the long-term complications prevalence in people with diabetes type 2 included: neuropathy 24.2%, nephropathy 12.5%, retinopathy 8.2%, amputation 0.7%, dialysis 0.6% and blindness 0.4% (Groos et al. 2017).

Asymmetric dimethylarginine (ADMA) is an endogenous competitive inhibitor of nitric oxide synthase (NOS), an enzyme responsible for the formation of NO. ADMA decreases NO production and leads to ED. Elevated levels of ADMA have been reported in atherosclerosis, hypercholesterolemia, hypertension, chronic renal failure, and diabetes (Tsikas et al. 2018). Major mechanisms that lead to increased ADMA in different diseases include oxidative stress, hyperhomocysteinemia, and high blood glucose concentrations (Wieczor et al. 2021). The results of a recent meta-analysis have shown that the use of ADMA as an ED biomarker in type 2 diabetes mellitus (T2DM) may help in early diagnosis, monitoring, and effective therapeutic management of the disease (Hisalkar et al. 2017). However, regarding ADMA in T2DM also conflicting reports exist. Earlier study (Krzyzanowska et al. 2006) has found that levels of plasma ADMA are associated with macrovascular disease independent of traditional cardiovascular risk factors and total homocysteine levels in patients with T2DM, whereas another study (Paiva et al. 2003) do not support these findings.

C-reactive protein (CRP) is a sensitive marker of systemic low-grade inflammation. Many authors believe that CRP is not only a marker but also an important mediator of inflammatory processes. However, its role in the human body, is still poorly understood. It has been shown that CRP is a

component of the innate immune system, which activates the classical complement pathway, binds to receptors on phagocytic immune cells, and stimulates cytokine production (Ghule et al. 2021). A recent meta-analysis has reported that T2DM risk is strongly associated with elevated levels of inflammatory cytokines, including CRP (Liu et al. 2016). However, in T2DM Chinese patients, increased CRP plasma levels were only positively associated in patients with high values of glycated hemoglobin (HbA1c), but not in those with low HbA1c values (Pan et al. 2017). Based on these findings, the authors have suggested that elevated CRP levels are merely a byproduct of hyperglycemia and not a causal risk factor for T2DM development.

Some of the studies have shown that ethnic background also influences the levels of both serum ADMA and CRP in the general population (Kelley-Hedgpeeth et al. 2008; Sydow et al. 2010). However, the impact of T2DM duration on serum ADMA and CRP concentrations among different ethnicities have not been extensively investigated. The aim of the present study was to assess the impact of T2DM duration on serum ADMA and CRP concentrations in Bosnian patients.

Material and Methods

Subjects. Participants for this cross-sectional study were randomly selected from the Out-Patient Family Medicine Clinic “Višnjik”, Sarajevo, Bosnia and Herzegovina. T2DM was defined by previously established criteria (American Diabetes Association 2005). Based on diabetes duration, patients with T2DM were divided into: up to 10-year diabetes duration (≤ 10 years T2DM) group and greater than 10-year diabetes duration (> 10 years T2DM) group. All T2DM patients were receiving antidiabetic and antihypertensive therapy. Some were receiving lipid lowering drugs and/or aspirin for at least 6 previous months. Exclusion criteria were the presence of sustained acute and chronic infections, malignancy, hepatic or renal disease, diabetic retinopathy and nephropathy and other endocrine dysfunction. The control group (CG) consisted of apparently healthy sex-matched subjects with no history of T2DM, endocrine dysfunction, hyperlipidemia, hypertension or coronary heart diseases. The study was approved by the Ethics Committee of the Medical Faculty at the University of Sarajevo and was performed in accordance with the Declaration of Helsinki.

Parameters evaluated. The blood was collected in the morning after overnight patients fasting.

Sampling was done without stasis using the vacutainer technique. Serum ADMA concentration was determined by ELISA method (machine STAT FAX 2100, USA) at the Department of Physiology and Biochemistry, Faculty of Medicine at the University of Sarajevo. An ADMA[®] - ELISA kit (DLD Diagnostika GmbH, Hamburg, Germany) was used as the reagent. Serum CRP was determined by particle-enhanced immunonephelometry with the use of BN II analyzer (Siemens Healthineers Global, Erlangen, Germany). CardioPhase high-sensitivity CRP (hs-CRP) (DADE BEHRING, Marburg, Germany) was used as a diagnostic reagent. Total cholesterol (TC), HDL-cholesterol (HDL-C), and triglycerides (TG) levels were measured using standard enzymatic methods. LDL-cholesterol (LDL-C) levels were calculated using the Friedewald formula (Friedewald et al. 1972). Fasting glucose (FG) level was determined by the glucose oxidase method. HbA1c was measured by immunoturbidimetric method (TINIA; Siemens Healthcare Diagnostics Ltd, Camberley, United Kingdom). All biochemical tests, except for ADMA determination, were performed at the Department of Clinical Chemistry and Biochemistry at the University of Sarajevo Clinical Center.

The height was measured with stadiometer (seca 213; seca[®]) and the weight with a digital scale (BS-03; Shenzhen J and E Electronic Co., Ltd). From these two measurements, body mass index (BMI) was calculated for each participant by dividing the weight in kilograms by height in square meters. Waist-to-hip ratio (WHP) was determined by measuring the narrowest part of the patient's waist and the widest part of the patient's hips and dividing these two measurements. Three supine blood pressure recordings were made after the patient rested for 5 min using an electronic blood pressure monitor (Omron 705c, Kyoto, Japan). The mean of the readings was used in this study. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg and prehypertension as SBP between 120 and 139 mmHg or DBP between 80 and 89 (Wang and Wang 2004).

Statistical analysis. SPSS version 17.0 (SPSS, Chicago, IL, USA) run in Windows was used to conduct the statistical analyses. Shapiro-Wilk normality test was used to test the distribution of variables. Normally distributed data are presented as mean $\pm$ standard deviation (SD) and skewed variables as median and interquartile ranges. ANOVA test followed by post-hoc Scheffe test or Kruskal-Wallis test followed by Man-Whitney test were used to compare the differences between groups, as

appropriate. The value $p < 0.05$ was considered statistically significant.

Results

This cross-sectional study included 38 patients in ≤ 10 years T2DM group, 22 patients in >10 years T2DM group, and 60 apparently healthy individuals (Table 1). Patients in the >10 years T2DM group were significantly older (64.00 ± 2.69 years) than patients in the ≤ 10 years T2DM group (55.07 ± 4.72 years) ($p < 0.001$) and the CG (52.25 ± 7.26 years) ($p < 0.001$). Patients in the >10 years T2DM group and the ≤ 10 years T2DM group had significantly ($p < 0.001$) larger/higher BMIs compared to CG patients. Patients in the >10 years T2DM group had significantly higher WHR values ($p < 0.05$) compared to patients in the ≤ 10 years T2DM group and the CG ($p < 0.001$). Statistically significant difference in WHR values was observed between the ≤ 10 years T2DM group and the CG ($p < 0.01$). SBP and DBP were significantly higher ($p < 0.001$) in the >10 years T2DM group compared to the CG. SBP was significantly higher ($p < 0.01$) in the ≤ 10 years T2DM group compared to the CG. Statistically significant difference ($p < 0.05$) in DBP values was observed between the >10 years T2DM group and the ≤ 10 years T2DM group (Table 1).

FG and HbA1c values were significantly higher ($p < 0.001$) in the >10 years T2DM group and in the ≤ 10 years T2DM group compared to the CG. HbA1c value was significantly higher ($p < 0.05$) in the >10 years T2DM group compared to the ≤ 10 years T2DM group. TC was significantly higher ($p < 0.01$) in the >10 years T2DM group and in the ≤ 10 years T2DM group compared to the CG. TG was significantly higher ($p < 0.001$) in the >10 years T2DM group and in the ≤ 10 years T2DM group compared to the CG. Patients in >10 years T2DM group had significantly higher TG ($p < 0.01$) compared to patients in the ≤ 10 years T2DM group. Patients in the >10 years T2DM group had significantly lower HDL-C levels compared to patients in the ≤ 10 years T2DM group ($p < 0.01$) and the CG ($p < 0.001$). HDL-C values between the ≤ 10 years T2DM group and the CG were significantly different ($p < 0.01$). LDL-C was significantly higher in the >10 years T2DM group ($p < 0.001$) and the ≤ 10 years T2DM group ($p < 0.01$) compared to the CG. Patients in the >10 years T2DM group had significantly higher LDL-C levels ($p < 0.01$) compared to patients in the ≤ 10 years T2DM group (Table 1). In the ≤ 10 years T2DM group and in the >10 years T2DM group HbA1c and CRP slightly correlated but the correlation was not statistically significant (data not shown).

Table 1
Baseline characteristics of study participants.

Variables	CG (n=60)	≤10 years T2DM group (n=38)	>10 years T2DM group (n=22)
Age (years)	52.25±7.26	55.07±4.72	64.00±2.69***
BMI (kg/m ²)	27.19 (25.12–28.71)	30.99 (28.70–32.88)***	31.48 (28.99–32.56)***
WHR	0.85±0.082	0.91±0.09**	0.97±0.05***
SBP (mmHg)	120.00 (110.00–140.00)	140.00 (125.00–150.00)**	150.00 (130.00–150.00)***
DBP (mmHg)	80.00 (75.00–85.00)	80 (80.00–90.00)	90.00 (80.00–100.00)***
FG (mmol/L)	5.10 (4.70–5.80)	7.65 (5.95–9.82)***	8.65 (6.57–10.42)***
HbA1c (%)	5.70 (5.40–5.80)	6.70 (6.20–7.65)***	7.60 (6.80–9.05)***
TC (mmol/L)	5.29±0.96	5.89±0.75**	6.16±0.89**
TG (mmol/L)	1.42 (1.07–1.92)	1.94 (1.55–2.87)***	3.16 (2.09–4.21)***
HDL-C (mmol/L)	1.39±0.29	1.19±0.27**	0.94±0.22***
LDL-C (mmol/L)	3.22±0.74	3.81±0.81**	4.69±0.85***

Data are presented as mean ± standard deviation (SD) or median and interquartile ranges. Data were analyzed by ANOVA test followed by post-hoc Scheffe test or Kruskal-Wallis test followed by Man-Whitney test. **p<0.01, ***p<0.001 vs. CG; *p<0.05, **p<0.01, ***p<0.001 vs. ≤10 years T2DM group. Abbreviations: CG – control group; BMI – body mass index; DBP – diastolic blood pressure; FG – fasting glucose; HbA1c – glycated hemoglobin; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; SBP – systolic blood pressure; T2DM – type 2 diabetes mellitus; TC – total cholesterol; TG – triglycerides; WHR – waist-hip ratio.

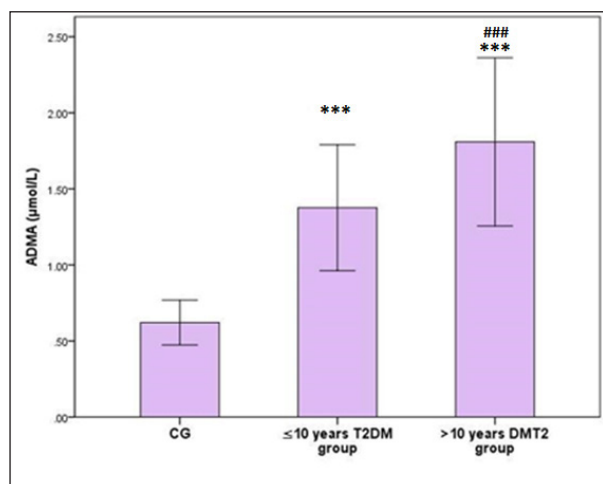


Figure 1. Differences in the serum asymmetric dimethylarginine (ADMA) concentrations between the tested groups. Data are presented as mean ± standard deviation (SD). Since variables had normal distribution, the difference between the groups was tested by ANOVA test followed by post-hoc Scheffe test. ***p<0.001 vs. CG; ***p<0.001 vs. ≤10 years T2DM group. Abbreviation: CG – control group; T2DM – type 2 diabetes mellitus.

One-way ANOVA investigating the effect of T2DM duration on serum ADMA concentration was found to be significant ($F=115.074$, $p<0.001$). Post-hoc Scheffe testing revealed significant difference in ADMA levels in-between all tested groups ($p<0.001$).

Serum ADMA concentration in the >10 years T2DM group (1.81 ± 0.15 μmol/L) was significantly higher compared to serum ADMA concentration in the ≤10 years T2DM group (1.38 ± 0.41 μmol/L; $p<0.001$) and in the CG (0.62 ± 0.15 μmol/L; $p<0.001$). A significant difference in serum ADMA concentration was observed between ≤10 years T2DM group and CG ($p<0.001$) (Figure 1).

Kruskal-Wallis test investigating the effect of T2DM duration on serum CRP concentration was found to be significant ($p<0.001$). Post-hoc Mann-Whitney U testing revealed significant difference in CRP levels in-between all tested groups ($p<0.001$). Serum CRP concentration in the >10 years T2DM group [5.95 (4.20 – 9.12) mg/L] was significantly higher compared to serum CRP concentration in the ≤10 years T2DM group [2.35 (1.40 – 4.30) mg/L; $p<0.001$] and in the CG [0.85 (0.50 – 1.30) mg/L; $p<0.001$]. A significant difference in serum CRP concentration was observed between the ≤10 years T2DM group and CG ($p<0.001$) (Figure 2).

Discussion

The present study is, to our knowledge, the first one assessing the impact of T2DM duration on the serum ADMA and CRP concentrations in Bosnian patients. The results of our investigation showed that patients in >10 years T2DM group had significantly higher levels of both ADMA and CRP compared to

patients in the ≤ 10 years T2DM ($p < 0.001$) group and the CG ($p < 0.001$).

The impact of the ethnic background on serum ADMA level, as an emerging cardiometabolic risk factor, is well documented in European population. An earlier study (Sydow *et al.* 2010) has shown that plasma ADMA concentration is different across various ethnic groups in the United States and correlates with age, BMI, and renal function in healthy older men and women. Although effect of sex on the serum ADMA levels has previously been reported, this finding was not confirmed in the above mentioned study sample. Higher ADMA values have been found in non-diabetic, non-obese Caucasian women compared to African women, whereas African and Caucasian men had similar levels of ADMA (Schutte *et al.* 2010). In the same study, positive association between ADMA and pulse wave velocity was found in the African men and to a lesser degree in the Caucasian women. The observed finding implies possible role of ADMA in the regulation of arterial stiffness. However, positive association between ADMA and insulin resistance in African, Caucasian or Hispanic participants has not been reported.

Newly diagnosed T2DM patients without cardiovascular complications have higher mean ADMA values as well as worse inflammatory and metabolic profile compared to healthy subjects. Moreover, positive correlation between ADMA and CRP values has been reported (Sciacqua *et al.* 2012). ADMA level has been found to be a strong predictor of coronary artery disease in T2DM patients (Jawalekar *et al.* 2013). Authors have suggested that ADMA directly disturbs NO-dependent vasodilatation and has mechanisms of actions that are independent of other risk factors for cardiometabolic diseases such as hypercholesterolemia, low-grade inflammation, hypertension, and smoking. Previous study has reported higher levels of ADMA in T2DM patients with macrovascular complications compared to T2DM patients without macrovascular complications and healthy controls. Major determinant of ADMA has been found to be total homocysteine suggesting important role of ADMA in pathogenesis of atherosclerosis. However, in the above mentioned study, correlation between ADMA and homocysteine was not observed, which might imply that mechanisms of ADMA action are independent of homocysteine (Celik *et al.* 2014). The recent study with the follow up of 6.5 years showed that elevated ADMA levels predict not only the incidence of macrovascular, but also microvascular complications in T2DM patients, which are further associated with age, hypertension, and renal

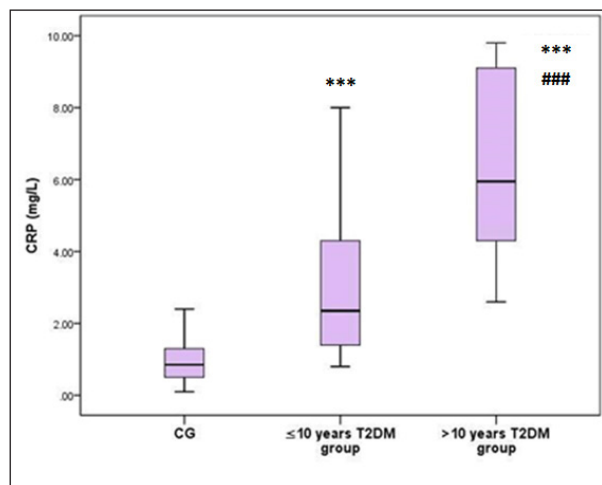


Figure 2. Differences in the serum C-reactive protein (CRP) concentrations between the tested groups. Data are presented as median and interquartile ranges. Since variables did not have normal distribution, the difference between the groups was tested by Kruskal-Wallis test followed by Man-Whitney test. *** $p < 0.001$ vs. CG; ### $p < 0.001$ vs. ≤ 10 years T2DM group. Abbreviations: CG – control group; T2DM – type 2 diabetes mellitus.

impairment (Ganz *et al.* 2017). Although the majority of studies have demonstrated that patients with T2DM have higher levels of ADMA, this finding was not uniformly reported, which suggests a possible role of certain confounding factors. Hence, it remains to be elucidated whether ADMA is a cause or a result of macro/microvascular disease in T2DM patients.

An association between ED and hyperglycemia in patients with T2DM is well established. However, the results of an earlier study have shown that plasma ADMA levels did not represent a significant independent risk factor for long-term adverse cardiovascular events in T2DM patients with good glycemic control (Hsu *et al.* 2014). The results of our study partly corroborate these findings since higher levels of both ADMA and HbA1c were observed in the > 10 years T2DM group compared to ≤ 10 years T2DM group, suggesting an increase of ADMA level with longer T2DM duration in patients with poor glycemic control. In contrast to our observations, previous human and animal studies (Xiong *et al.* 2005) did not establish a relationship between T2DM duration and serum ADMA concentration. Nevertheless, the authors did report higher ADMA values in T2DM patients with macroangiopathy compared to those without. The authors have suggested that this finding could be explained by a poor glycemic control. Since ADMA is a marker of ED, which

contributes to the development of T2DM vascular complications, measurement of serum ADMA levels at different stages of T2DM duration could assist in the monitoring and treatment of ED in T2DM.

The highest levels of CRP have been found in African-Americans, followed in order by Hispanic, Caucasian, Chinese, and Japanese middle-aged women (Matthews et al. 2005). The above-mentioned study aimed to assess cardiovascular risk factors in 2834 premenopausal women and to evaluate whether lifestyle, education level, and geographic location might explain the extent of ethnic differences in the risk. Their findings have shown that the majority, although not all, of the ethnic differences may be explained by geographic place and socioeconomic status, whereas the largest ethnic differences, among other risk factors, were seen at the levels of hs-CRP. The results of an earlier study (Kelley-Hedgpeeth et al. 2008) have demonstrated that modifiable cardiometabolic risk factors, especially BMI, account for much but not all of the ethnic differences at the levels of CRP. Thus, impact of ethnic background on CRP levels remains unclear and requires further investigation.

An association between elevated levels of CRP and an increased risk of T2DM has been reported by several meta-analyses (Wang et al. 2013; Liu et al. 2016). Findings from the prospective studies that were included in these meta-analyses have pointed to complex interactions between development of T2DM and inflammation. It should be noted that chronic systemic low-grade inflammation is a possible culprit in the pathogenesis of T2DM and not the acute inflammation. Although these findings suggest that T2DM might act as an autoinflammatory disease, further research is warranted to fully understand CRP's potential causal role in the development of T2DM. The results from meta-analyses suggest that increased levels of CRP are associated with the elevated risk of T2DM. Therapeutic measures that have been shown to reduce CRP should be implemented in everyday clinical practice.

The results of our study showed that patients in the >10 years T2DM group had significantly higher levels of CRP compared to patients in ≤10 years T2DM group ($p < 0.001$), suggesting an increase in CRP with T2DM progression. These findings might be explained by elevated HbA1c in the >10 years T2DM group compared to the ≤10 years T2DM group, implying that increased CRP levels might be a consequence of hyperglycemia as it has been proposed in study of Pan et al. (2017). However, results of our study did not show correlation between

HbA1c and CRP in the ≤10 years T2DM group and the >10 years T2DM group, which might be due to a small study sample. Furthermore, patients in the >10 years T2DM group had higher WHR and LDL-C and lower HDL-C values than patients in the ≤10 years T2DM group, suggesting an association between CRP and cardiometabolic syndrome components in patients with T2DM as it has been demonstrated by Kanmani et al. (2019). Our findings support the inclusion of hs-CRP measurement in the assessment of cardiometabolic syndrome in patients with T2DM.

Results of the current study did not show significant difference in the distribution of male and female participants within different experimental groups. Many studies indicate that female diabetics are at greater CVD risk and mortality than male ones. Results of the previous study (Zaciragic et al. 2014) have shown higher values of serum ADMA and CRP concentration in female compared to male patients with T2DM. The obtained results suggested that more pronounced endothelial dysfunction and chronic low-grade inflammation in diabetic women compared to diabetic men could be one of the possible explanations for increased cardiovascular morbidity and mortality in female patients with T2DM.

An impact of ethnicity on serum ADMA and CRP concentration has been reported. Accordingly, results of the ASOS and African-PREDICT studies have shown that serum ADMA concentration is higher in white than in black boys. However, this difference disappears in adulthood. ADMA metabolism excretion was lower in the black in comparison to the white subjects suggesting ethnicity-dependent renal and hepatic elimination of ADMA in the childhood (Bollenbach et al. 2020). As for the CRP, earlier systematic review of population-based studies has demonstrated that in adults, non-white race and increasing poverty were associated with increased CRP values (Nazmi and Victora 2007).

The main outcome of the present study is that it established the impact of T2DM duration on serum ADMA and CRP concentration in Bosnian patients. Nevertheless, our study is limited by its small sample size and cross-sectional design, which did not allow us to draw any causal conclusions regarding this relationship. Moreover, obtained results should be interpreted with caution since patients in the >10 years T2DM group were significantly older than patients in the ≤10 years T2DM group and in the CG. Thus, future studies should try to avoid this potential source of bias. However, these results have clinical implications, because they point to progression of ED and systemic low-grade inflammation with

prolonged T2DM duration. Since both of these conditions were linked with diabetic complications, the findings presented in this study imply the necessity of implementing of aggressive treatment paradigms in lowering both ADMA and CRP levels in T2DM patients.

Conclusion

The present study demonstrated an increase in ADMA and CRP serum concentrations with

advancement of T2DM. Although the measurement of these two markers is not a part of the standard T2DM follow-up, these results suggest that both ADMA and CRP might serve as indicators of the endothelial dysfunction and chronic systemic low-grade inflammation progression in patients with T2DM. However, further larger prospective studies are required to confirm these findings.

Conflict of interest: *The authors declare no conflict of interest.*

References

- American Diabetes Association. Standards of medical care for patients with diabetes mellitus (Position Statement). *Diabetes Care* 28 (suppl 1), s4–s36, 2005.
- Baron AD, Brechtel G. Insulin differentially regulates systemic and skeletal muscle vascular resistance. *Am J Physiol* 265, E61–E67, 1993.
- Bollenbach A, Schutte AE, Kruger R, Tsikas D. An ethnic comparison of arginine dimethylation and cardiometabolic factors in healthy black and white youth: The ASOS and African-PREDICT Studies. *J Clin Med* 9, 844, 2020.
- Celik M, Cerrah S, Arabul M, Akalin A. Relation of asymmetric dimethylarginine levels to macrovascular disease and inflammation markers in type 2 diabetic patients. *J Diabetes Res* 2014, 139215, 2014.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18, 499–502, 1972.
- Ganz T, Wainstein J, Gilad S, Limor R, Boaz M, Stern N. Serum asymmetric dimethylarginine and arginine levels predict microvascular and macrovascular complications in type 2 diabetes mellitus. *Diabetes Metab Res Rev* 33, 2017.
- Ghule A, Kamble TK, Talwar D, Kumar S, Acharya S, Wanjari A, Gaidhane SA, Agrawal S. Association of serum high sensitivity C-reactive protein with pre-diabetes in rural population: A two-year cross-sectional study. *Cureus* 13, e19088, 2021.
- Groos S, Kretschmann J, Macare C, Weber A, Hagen B. Quality assurance report 2016 disease-management-programmes in North Rhine, 2017. Available at https://www.kvno.de/downloads/quali/qualbe_dmp16.pdf. Accessed 23 Jun 2022.
- Hisalkar PJ, Shekhar PB, Bidwe SE, Kamble CG, Nemade ST, Powar JD, Patne A. Asymmetrical dimethylarginine (ADMA), an endothelial dysfunctional biomarker for type 2 diabetes mellitus, the meta analysis. *Ind J Bas Appl Med Res* 6, 496–506, 2017.
- Hsu CP, Hsu PF, Chung MY, Lin SJ, Lu TM. Asymmetric dimethylarginine and long-term adverse cardiovascular events in patients with type 2 diabetes: relation with the glycemic control. *Cardiovasc Diabetol* 13, 156, 2014.
- Jawalekar SL, Karnik A, Bhutay A. Risk of cardiovascular diseases in diabetes mellitus and serum concentration of asymmetrical dimethylarginine. *Biochem Res Int* 2013, 189430, 2013.
- Kanmani S, Kwon M, Shin MK, Kim MK. Association of C-reactive protein with risk of developing type 2 diabetes mellitus, and role of obesity and hypertension: A large population-based Korean cohort study. *Sci Rep* 9, 4573, 2019.
- Kelley-Hedgpeeth A, Lloyd-Jones DM, Colvin A, Matthews KA, Johnston J, Sowers MR, Sternfeld B, Pasternak RC, Chae CU; SWAN Investigators. Ethnic differences in C-reactive protein concentrations. *Clin Chem* 54, 1027–1037, 2008.
- Krzyzanowska K, Mittermayer F, Schnack C, Hofer M, Wolzt M, Schernthaner G. Asymmetric dimethylarginine is associated with macrovascular disease and total homocysteine in patients with type 2 diabetes. *Atherosclerosis* 189, 236–240, 2006.
- Kuniss N, Freyer M, Muller N, Kielstein V, Muller UA. Expectations and fear of diabetes-related long-term complications in people with type 2 diabetes at primary care level. *Acta Diabetol* 56, 33–38, 2019.
- Liu C, Feng X, Li Q, Wang Y, Li Q, Hua M. Adiponectin, TNF- α and inflammatory cytokines and risk of type 2 diabetes: A systematic review and meta-analysis. *Cytokine* 86, 100–109, 2016.

- Matthews KA, Sowers MF, Derby CA, Stein E, Miracle-McMahill H, Crawford SL, Pasternak RC. Ethnic differences in cardiovascular risk factor burden among middle-aged women: Study of Women's Health Across the Nation (SWAN). *Am Heart J* 149, 1066–1073, 2005.
- Nazmi A, Victora CG. Socioeconomic and racial/ethnic differentials of C-reactive protein levels: a systematic review of population-based studies. *BMC Public Health* 7, 212, 2007.
- Pan A, Wang Y, Yuan JM, Koh WP. High-sensitive C-reactive protein and risk of incident type 2 diabetes: a case-control study nested within the Singapore Chinese Health Study. *BMC Endocr Disord* 17, 8, 2017.
- Paiva H, Lehtimäki T, Laakso J, Ruokonen I, Rantalaiho V, Wirta O, Pasternack A, Laaksonen R. Plasma concentrations of asymmetric-dimethyl-arginine in type 2 diabetes associate with glycemic control and glomerular filtration rate but not with risk factors of vasculopathy. *Metabolism* 52, 303–307, 2003.
- Rajendran P, Rengarajan T, Thangavel J, Nishigaki Y, Sakthisekaran D, Sethi G, Nishigaki I. The vascular endothelium and human diseases. *Int J Biol Sci* 9, 1057–1069, 2013.
- Sciacqua A, Grillo N, Quero M, Sesti G, Perticone F. Asymmetric dimethylarginine plasma levels and endothelial function in newly diagnosed type 2 diabetic patients. *Int J Mol Sci* 13, 13804–13815, 2012.
- Schutte AE, Schutte R, Huisman HW, van Rooyen JM, Fourie CM, Malan L, Malan NT, Schwedhelm E, Strimbeanu S, Anderssohn M, Boger RH. Dimethylarginines: their vascular and metabolic roles in Africans and Caucasians. *Eur J Endocrinol* 162, 525–533, 2010.
- Shi Y, Vanhoutte PM. Macro- and microvascular endothelial dysfunction in diabetes. *J Diabetes* 9, 434–449, 2017.
- Sydow K, Fortmann SP, Fair JM, Varady A, Hlatky MA, Go AS, Iribarren C, Tsao PS; ADVANCE Investigators. Distribution of asymmetric dimethylarginine among 980 healthy, older adults of different ethnicities. *Clin Chem* 56, 111–120, 2010.
- Tsikas D, Bollenbach A, Hanff E, Kayacelebi AA. Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and homoarginine (hArg): the ADMA, SDMA and hArg paradoxes. *Cardiovasc Diabetol* 17, 1, 2018.
- Wang X, Bao W, Liu J, Ouyang YY, Wang D, Rong S, Xiao X, Shan ZL, Zhang Y, Yao P, Liu LG. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. *Diabetes Care* 36, 166–175, 2013.
- Wang Y, Wang QJ. The prevalence of prehypertension and hypertension among US adults according to the new joint national committee guidelines: New challenges of the old problem. *Arch Intern Med* 164, 2126–2134, 2004.
- Wieczor R, Wieczor AM, Kulwas A, Rosc D. ADMA (asymmetric dimethylarginine) and angiogenic potential in patients with type 2 diabetes and prediabetes. *Exp Biol Med* 246, 153–162, 2021.
- Xiong Y, Lei M, Fu S, Fu Y. Effect of diabetic duration on serum concentrations of endogenous inhibitor of nitric oxide synthase in patients and rats with diabetes. *Life Sci* 77, 149–159, 2005.
- Zaciragic A, Hasanefendic B, Avdagic N, Lepara O, Babic N, Huskic J, Mulabegovic N. Gender comparison of serum asymmetric dimethylarginine and C-reactive protein concentration in patients with diabetes mellitus type 2. *Medical Journal* 20, 85–90, 2014.