Plasma Asymmetric Dimethylarginine (ADMA) is Associated with Retinopathy in Type 2 Diabetes Mellitus.

Maciej T. Malecki M.D., Ph.D. 1*, Anetta Undas M.D., Ph.D. 2*, Katarzyna Cyganek M.D., Ph.D. 1, Barbara Mirkiewicz-Sieradzka M.D., Ph.D. 1, Paweł Wolkow M.D., Ph.D. 3, Grzegorz Osmenda M.D., Ph.D. 3, Małgorzata Walus-Miarka M.D., Ph.D. 1, Tomasz J. Guzik M.D., Ph.D. 3, Jacek Sieradzki M.D., Ph.D. 1

1Metabolic Diseases, Jagiellonian University, Krakow, Poland
2Institute of Cardiology, Jagiellonian University, Krakow, Poland
3Pharmacology, Jagiellonian University, Krakow, Poland
*these authors equally contributed to this work

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Correspondence:
Maciej T. Malecki, M.D., Ph.D.
Metabolic Diseases, Jagiellonian University,
15 Kopernika Street, 31-501 Krakow, Poland
e-mail: malecki_malecki@yahoo.com

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Decreased availability of nitric oxide (NO) that contributes to the development of diabetic vascular complications [1] is partially related to asymmetric dimethylarginine (ADMA). ADMA is an endogenous NO synthase inhibitor [2] and a competitive inhibitor of the cellular L-arginine up-take [3]. ADMA has been associated with atherosclerosis in non-diabetic populations [4] and with diabetic nephropathy in T1DM [5]. In humans, the stereoisomer of ADMA, symmetric dimethylarginine (SDMA), is produced in equivalent quantities; although, it does affect NO synthesis, SDMA may compete with arginine for cellular uptake [6].

Our objective was to evaluate the relationship between plasma ADMA and DR in type 2 diabetes mellitus (T2DM).

**RESEARCH DESIGN AND METHODS**

We examined 182 T2DM consecutive patients (mean age at examination 56.2±6.5 years). The previously described inclusion criteria were used [7]. Biochemical measurements were done using standard methods. Creatinine clearance was calculated according to the Cockroft-Gault formula. The control group consisted of 52 apparently healthy individuals matched for age and gender (mean age 54.5±7.1 years). This study was approved by the local Ethical Committee.

All T2DM patients underwent ophthalmological evaluation. Color fundus photographs were taken as previously described [8]. The final DR diagnosis was based on both ophthalmoscopy and photography [9, and on-line-only appendix]. The patients were assigned to one of three groups: a) no DR; b) non-proliferative DR; c) proliferative DR.

Plasma ADMA, SDMA, and L-arginine levels were measured by high-performance liquid chromatography [10].

We used logistic regression to study the association of ADMA, SDMA, and L-arginine with DR and the multivariable logistic regression to analyze whether clinical variables (gender, age of examination, duration of T2DM, HbA1c, arterial hypertension, lipids, BMI, and creatinine clearance) modify the odds of DR occurrence. Variables independently associated with DR were identified using a stepwise selection procedure. A p-value<0.05 was considered statistically significant. Model fit was evaluated using Bayesian Information Criterion.

**RESULTS**

Non-proliferative DR was diagnosed in 68 (37.3%) and proliferative DR in 3 (1.6%) subjects, respectively. Both DR groups were combined for the further analyses. The characteristics of T2DM patients with DR and without it (NDR) are available in the on-line-only appendix table 1.

The ADMA level was highest in T2DM patients with DR (0.60±0.06 µmol/L), intermediate in NDR subjects (0.51±0.06 µmol/L), and lowest among the controls (0.45±0.05 µmol/L) (p<0.001 for all comparisons). Similarly, SDMA was higher in DR than in the NDR group (0.45±0.06 µmol/L vs. 0.41±0.06, p<0.001) and lower among the controls than in both diabetic groups (0.36±0.06 µmol/L, p<0.001 for both comparisons). In contrast, L-arginine levels were similar in diabetic groups (0.80±0.14 vs. 0.79±0.13 µmol/L, respectively p=0.38), however, they were higher compared with the control group (0.64±0.08 µmol/L, p<0.0001) (Figure 1). Levels of ADMA, SDMA and L-arginine were significantly correlated.

In search of a potential association between the three studied analytes and DR, we checked whether their combination could form a better predictor than each of them separately. L-Arginine, both in combination and alone, was not associated with DR. However, SDMA was associated with DR in univariate analysis (OR=1.12, 95%CI:1.06–1.18, p<0.0001) but not when both ADMA and SDMA were incorporated into the model (p=0.35). ADMA was the only variable consistently associated with DR, both in univariate analysis (OR=1.39, 95%CI:1.26–1.53, p<0.0001) and in combination with the other two analytes
Asymmetric dimethylarginine level and diabetic retinopathy (OR=1.44, 95%CI:1.28–1.61, \( p<0.0001 \)). Thus, we used ADMA in the modeling in further analyses.

In a multivariable logistic regression with stepwise variable selection, plasma ADMA level was selected as a predictor of the presence of DR (\( p<0.0001 \), OR=1.80, 95%CI:1.47–2.19) in T2DM patients, in addition to the age at examination (\( p=0.0048 \), OR=0.85, 95%CI:0.75–0.95), creatinine clearance (\( p=0.0116 \), OR=1.24, 95%CI:1.05–1.46), and insulin therapy (\( p=0.0017 \), OR=7.0, 95%CI:2.08–23.61). Studied variables were not independent. Not unexpectedly, ADMA concentration, creatinine clearance and age at examination were all correlated to each other with ADMA positively correlated with age at examination (\( r=0.5 \), \( p<0.0001 \)) and negatively correlated with creatinine clearance (\( r=-0.4 \), \( p<0.0001 \)).

ADMA remained also (\( p<0.0001 \), OR=1.77, 95%CI:1.44–2.17) a significant, independent predictor of the presence of DR in a broader multivariable model with forced inclusion of well-proven risk factors of DR into a regression equation (On-line-only appendix table 2).

CONCLUSIONS

This is the first report suggesting an association between an elevated circulating ADMA level and DR in T2DM. Elevated ADMA levels in aqueous humor were recently described in patients with severe DR [11]. In T1DM, there was no difference in plasma ADMA levels between patients with DR and those free of it [5]. The reasons for this discrepancy might be, at least in part, associated with resistance to insulin action present in T2DM. There is evidence that ADMA may be both a result and a cause of insulin resistance [6]. Interestingly, several reports indicate that resistance to insulin plays a role in the pathogenesis of DR [12]. Thus, one might speculate that the putative ADMA role in the pathogenesis of DR is more prominent in T2DM than in T1DM.

There is evidence that increased ADMA is typical of chronic renal failure. Thus, one might expect that high ADMA levels in DR subjects reflects a phenomenon of the coexistence of DR and diabetic nephropathy [13]. The mean creatinine levels were, however, within the normal range in both diabetic groups. An unexpected apparent protected effect of age at examination and decreased creatinine clearance require a comment. In the presence of co-linear covariates, modeling is often a trade-off between a biased estimate with relatively high precision (when co-linear covariates are removed from the model) and a less biased estimate with lower precision (with co-linear covariates included) [14]. It is, however, important to note that in a stepwise selection or in forced inclusion models that contained creatinine clearance and/or age at examination, association of ADMA with retinopathy became even stronger.

The potential limitation of our study is that ADMA was measured at a single time point. Thus, it did not answer the question whether elevated ADMA levels stimulate the development of DR or if they merely constituted its marker. Prospective studies are necessary to clarify this.
References:

Figure 1. Concentrations of ADMA, SDMA and arginine (µmol/L) in the studied groups: –
control subjects (CTRL), type 2 diabetes mellitus patients without retinopathy (NDR), and
type 2 diabetes mellitus patients with retinopathy (DR) are shown. Data are shown as range
(whiskers) and 25, 50, and 75 percentiles (boxes).