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Association of Plasma ADMA Levels with MRI Markers of Vascular Brain Injury: The Framingham Offspring Study

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Abstract

Background and Purpose—Asymmetric dimethylarginine (ADMA), an inhibitor of endothelial nitric oxide synthase, is a marker of endothelial dysfunction. Elevated circulating ADMA concentrations have been associated with systemic and carotid atherosclerosis, an elevated risk of developing stroke and with MRI white matter hyperintensities (WMH). The relation of plasma ADMA to subclinical vascular brain injury has not been previously studied in a middle-aged, community-based sample.

Methods—In 2013 stroke-free Framingham Offspring (mean age 58±9.5yrs, 53% women) we related baseline plasma ADMA levels (1995–98) to subsequent brain MRI (1999–2004) measures of subclinical vascular injury: presence of silent brain infarcts (SBI) and large white matter hyperintensity volume (LWMH; defined as >1SD above age-specific mean).

Results—Prevalence of SBI and LWMH were 10.7% and 12.6%, respectively. In multivariable analyses adjusting for age-, sex- and traditional stroke risk factors, higher ADMA levels were associated with an increased risk of prevalent SBI (OR per SD increase in ADMA: 1.16, 95%CI: 1.01–1.33, p= 0.04).We observed that participants in the upper three age-specific quartiles of plasma ADMA had an increased prevalence of SBI (OR for Q2–4 versus Q1:1.43, 95%CI: 1.00–2.04, p<0.05). Prevalence of SBI in Q1and Q2–4 was 8.3% and 11.6%, respectively. Prevalence of LWMH did not differ according to ADMA concentrations.

Conclusion—Higher plasma ADMA was associated with increased prevalence of SBI after adjustment for traditional stroke risk factors. Thus, ADMA may be a potentially useful new biomarker

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of subclinical vascular brain injury, which is an important correlate of vascular cognitive impairment and risk of stroke.

Keywords

ADMA; endothelial dysfunction; silent cerebral infarct

Nitric oxide (NO) is a key mediator of normal endothelial function, also called the "endogenous anti-atherosclerotic molecule". Endothelial dysfunction is emerging as a marker of preclinical atherosclerosis.[1] Asymmetric dimethylarginine (ADMA) is a major endogenous inhibitor of endothelial nitric oxide synthase (eNOS), whereas its symmetric isomer, symmetric dimethylarginine (SDMA), does not inhibit eNOS. Accumulation of ADMA results in decreased NO bioavailability, supporting a pro-atherogenic role for ADMA.[2] Elevated plasma ADMA concentrations have been associated with the presence of numerous traditional and novel stroke risk factors such as hypertension,[3] diabetes,[4] left ventricular hypertrophy,[2,5] coronary artery disease (CAD),[6] atrial fibrillation (AF),[7] hypercholesterolemia,[8] hyperhomocysteinemia,[9] as well as with an increased risk of clinical and subclinical carotid and systemic atherosclerosis, and intimal-medial thickness (IMT).[10,11] In case-control studies, elevated ADMA concentrations were associated with an increased risk of developing stroke[12] and transient ischemic attack (TIA),[13]and recently with MRI findings of small vessel disease, specifically with severity of leukoariosis.[14]

White matter hyperintensities (WMH) and silent brain infarcts (SBI) are MRI abnormalities strongly related to stroke risk factors[15] and clinical stroke[16], and are accepted indicators of subclinical macro- and microvascular brain injury[15] Although the pathogenesis of these abnormalities is not fully understood, a diffuse arteriopathy affecting the deep small perforating cerebral vessels has been proposed as a mechanism underlying chronic ischemia, ischemic demyelination, axonal loss and gliosis.[17,18] Various vascular risk factors comprising the Framingham Stroke Risk Profile (FSRP),[15] circulating concentrations of inflammatory markers,[19] and homocysteine[20] have each been related to these MRI measures of subclinical vascular brain injury. However, the relation of ADMA, a putative novel biomarker of stroke risk, to subclinical vascular brain injury has not been studied in a community-based sample. Thus, we related plasma ADMA and SDMA levels to MRI measures of subclinical vascular brain injury in our middle–aged, community sample.

METHODS

Study Sample

The Framingham Study is a community-based, ongoing cohort study. In 1971, the offspring of the Original Cohort and their spouses were enrolled in the Framingham Offspring Study.[21] These participants have been assessed once every four years with medical histories, physical examinations and laboratory tests, and are currently undergoing their 9th examination. All persons have been under continuous surveillance for incident stroke and TIA. A total of 3,532 Offspring survivors attended the 6th Offspring examination (1995–1998), and blood drawn at this examination was utilized for ADMA estimation in 3,453 participants. All participants were invited to participate in brain MRI. A subset of 2,072 participants, who did not have known contraindications to MRI, underwent volumetric brain MRI between 1999 and 2004. From this subset, we excluded 59 persons with prevalent stroke, dementia or other neurological illness (such as multiple sclerosis or brain tumor) that could affect MRI measurements.[22] Although plasma ADMA and SDMA levels were not measured at the same time when brain MRI was performed; MRI changes of interest (SCI and WMH) accumulate over time, hence prior exposure to elevated levels of these markers are more likely to be related to MRI markers of subclinical disease, as previously shown for the association of plasma tHcy with silent brain

infarcts.[20] Our study sample comprises 2013 persons (53% female), with a mean age of 58±9.5 years. This study was approved by the Institutional Review Board at Boston University Medical Center and the Ethics Committee of the Hamburg Board of Physicians. All participants gave informed consent.

Measurement of Plasma ADMA and SDMA

Plasma ADMA and SDMA concentrations were measured on stored samples using liquid chromatography tandem mass-spectroscopy (LC-MS) techniques. The details of the assay and its excellent reproducibility with the coefficient of variation, 3.2% and 3.4% for ADMA and SDMA respectively, have been described in prior publications.[23] While SDMA is primarily eliminated by renal excretion, ADMA is mainly metabolized in kidneys by dimethylarginine dimethylaminohydrolases (DDAH) to citrulline and dimethylamine.

Brain Imaging

MRI acquisition and measurement techniques and inter-rater reliability have been detailed previously.[15,22] Briefly, the images were acquired on a 1 or 1.5 Tesla Siemens Magnetom and transferred to the centralized reading center at the University of California-Davis Medical center. The images were analyzed by operators blinded to the participant's identity, age, sex, plasma ADMA level and their exposure to stroke risk factors. Brain volume was determined by manual demarcation of the intracranial vault to establish the total cranial volume (TCV) followed by subsequent mathematical modeling to determine parenchymal volumes. The volume of abnormal white matter hyperintensity (WMH) was determined using previously described semi-automated methods.[22] WMH volume was corrected for a measure of head size, the total intracranial volume (TCV). Subjects were categorized as having extensive WMH if the log-WMH volume/TCV was more than 1 SD above the age-specific mean in this cohort. The presence or absence of an MRI infarct was determined manually by the operator, based on the size (≥3 mm), location and imaging characteristics of the lesion. The lesion was required to have CSF density on the subtraction image; if embedded within basal ganglia, it was further required to be distinctly isolated from the circle of Willis vessels to avoid confusion with dilated Virchow spaces. MRI infarcts were classified as silent brain infarcts (SBI) if the person had not had a clinically documented stroke at any time prior to MRI. All images were evaluated by three different raters with Kappa values for agreement ranging between 0.73 and 0.90.[22]

Definition of Covariates

The components of previously described and validated Framingham Stroke Risk Profile (FSRP) were used as baseline covariates.[24] The FSRP provides an estimate of the 10-year risk of stroke for a given subject, based on age, sex and measurements of several cardiovascular risk factors including systolic blood pressure (SBP), antihypertensive therapy, diabetes, smoking status, history of cardiovascular disease (CVD), and the presence of atrial fibrillation (AF). Blood pressure was recorded as the average of two physician-recorded measurements. Diabetes mellitus was defined by a recorded fasting blood glucose ≥126 mg/dl (7mmol/L), a previous diagnosis of diabetes mellitus, or the use of oral hypoglycemic agents or insulin. Smoking status was categorized as 'current smoker' or 'current non-smoker'. Prior CVD events included a diagnosis of coronary heart disease, congestive heart failure or peripheral arterial disease. The diagnosis of AF was based on electrocardiographic documentation of the arrhythmia on a standard 12-lead EKG obtained at the Heart Study examination, or elsewhere. Additional covariates in our analysis were serum creatinine, measured by modified Jaffe method, and plasma total homocysteine (tHcy), which was estimated using a previously validated method.[25] Plasma total homocysteine (tHcy) levels influence plasma ADMA levels through an inhibition of dimethylaminohydrolase (DDAH), an enzyme that regulates nitric oxide synthase (NOS) and hydrolyses ADMA[26] and have been associated with the risk of SBI.

[20] All covariates were measured at the same time that blood was drawn for ADMA and SDMA measurements.

Statistical analysis

We used multivariable logistic regression models to examine the cross-sectional associations between plasma ADMA or SDMA (independent variable) and MRI phenotypes (SBI and WMH; dependent variables) adjusting for known covariates influencing these variables. Plasma ADMA and SDMA were analyzed as continuous and as categorical variables using age-specific quartiles, defined within 10-year age groups. We additionally examined the trend across quartiles of plasma ADMA (or SDMA) for the MRI phenotypes, and also compared persons in the upper three quartiles to those in the lowest quartile. In initial analyses, we adjusted only for age, sex and time-interval between ADMA and SDMA, and MRI measures. We next additionally adjusted for components of the FSRP score. Finally, to explore whether the effect of ADMA was independent of serum creatinine and plasma tHcy levels, and use of statin and antithrombotic therapies, we individually adjusted for each in separate models. We did not adjust for carotid IMT or carotid stenosis as these phenotypes may lie along the causal pathway between ADMA concentrations and vascular brain injury. All analyses were performed using Statistical Analyses System (SAS® Institute Inc).

RESULTS

Table 1 presents the age-specific cut-points for each of the 4 quartiles of plasma ADMA in our sample. The distributions of demographic and vascular risk factor characteristics and other covariate data recorded at the baseline examination, as well as prevalence of SCI and LWMH are summarized in Table 2. In our sample, men were more likely to have higher mean systolic blood pressure, to be taking antihypertensive medication, to have diabetes, to have a history of cardiovascular disease and atrial fibrillation, and higher plasma tHcy and serum creatinine levels. Plasma ADMA and tHcy levels were correlated (Spearman correlation coefficient was 0.10; $p<0.001$). The prevalence of SBI and LWMH were 10.7% and 12.6%, respectively.

In Table 3 we present results of multivariable analysis relating plasma ADMA (modeled as a continuous and as a categorical variable) to the prevalence of SBI and WMH on brain MRI. In the initial analysis, ADMA concentrations were associated with an increased risk of prevalent SBI (OR per SD increase in ADMA: 1.15; 95%CI: 1.00–1.32, p= 0.04). Across ADMA quartiles, we observed trend across ADMA quartiles, such that participants in the upper three age-specific quartiles of plasma ADMA concentrations had an increased prevalence of SBI compared to persons in the lowest quartile (OR for Q2–4 vs. Q1: 1.46, 95%CI: 1.02–2.07, p=0.04). When further adjusted for traditional stroke risk factors, this association remained robust (OR per 1SD increment in ADMA: 1.16; 95%CI: 1.01–1.33, p=0.04), and was still significant across ADMA quartiles (OR for Q2–4 vs. Q1:1.43 95%CI: 1.00–2.04, $p\leq0.05$). Association between plasma ADMA concentration and increased prevalence of SBI was present after adjusting for use of statin and antithrombotic therapies (OR per 1SD increase in ADMA: 1.15; 95%CI: 1.00–1.32, p=0.04). Moreover, after adjustment for serum creatinine (OR per 1SD increased in ADMA: 1.15; 95%CI: 1.00–1.33, $p\leq0.05$) or for plasma tHcy concentrations (OR for Q2–4 vs. Q1:1.43, 95%CI: 1.00–2.04, $p\leq0.05$), the association remained significant. Prevalence of SBI in Q1 and Q2–4 was 8.3% and 11.6%, respectively. Prevalence of LWMH did not differ according to ADMA concentrations in any of the analyses. In addition, we did not observe significant association between plasma SDMA concentrations and the brain MRI measures of subclinical vascular brain injury (data presented in the supplement Table 4).

DISCUSSION

In the community-based, middle-aged Framingham Offspring study sample we observed an independent, cross-sectional association between higher plasma ADMA levels and increased prevalence of SBI, even after adjustment for traditional risk factors. Our findings demonstrate that plasma ADMA levels may be related not only to the risk of clinical stroke as suggested by prior studies, but also to the risk of subclinical vascular brain injury. Plasma SDMA was not associated with SBI or WMH.

ADMA, SDMA and monomethyl L-arginine (L-NMMA) are methylarginines produced during the degradation of methylated proteins. ADMA and L-NMMA are both competitive inhibitors of eNOS, but circulating levels of L-NMMA are low suggesting that ADMA is the major endogenous inhibitor of eNOS.[8,26,27] Accumulation of ADMA would thus decrease NO production. ADMA, a reasonably stable biomarker, may therefore be a true causal risk factor for endothelial dysfunction.[8] However, it could be merely a risk marker as it has been associated with numerous traditional and novel vascular risk factors such as plasma tHcy levels,[3–10] and with subclinical cerebrovascular disease.[14] In a small double-blind, vehicle-controlled study of 20 healthy subjects intravenously administered ADMA reduced vessel compliance and decreased cerebral blood flow measured using perfusion MRI.[28] This interesting observation suggests that circulating levels of ADMA may impact the pathogenesis of subclinical and/or clinical cerebrovascular disease, by influencing cerebral autoregulation and arterial vasomotor reactivity; this effect may also be mediated by NO pathways.

Although, the pathogenesis of cerebral SVD (small vessel disease; lacunar infarcts and leukoaraiosis) remains poorly understood, several studies suggested that chronic endothelial dysfunction has a significant role in mediating impaired cerebral autoregulation, and could contribute to the breakdown of the blood–brain barrier, which has been observed in small cerebral vessel dysfunction.[29] As already mentioned, studies have suggested that cerebral SVD includes a clinico-pathological and radiological spectrum with different underlying mechanisms contributing to lacunar infarcts on one hand, and to diffuse white-matter injury on the other. Chronic endothelial dysfunction of small perforating cerebral vessels could result in poor white matter irrigation and accumulation of white matter injury[30] even in the absence of clinical events. Silent brain infarcts (SBI) are defined as vascular brain lesions confirmed by CT or MRI, but without an apparent clinical correlate, and most of them (74–86%) are lacunar infarcts.[31] SBI have been associated with an increased risk of subsequent clinical stroke, and with cognitive impairment and memory loss.[32,33] Several vascular risk factors increase the risk of SBI including age, hypertension, diabetes mellitus, smoking and atrial fibrillation, as well as novel biomarkers, such as plasma homocysteine and serum cholesterol.[31, 34] However, prior studies have not examined the association of circulating ADMA and MRI-defined SBI.

Diffuse white-matter injury called ‘leukoaraiosis’, is a descriptive term referring to a radiological finding of diffuse, bilateral periventricular white matter hyperintensities (WMH) are a consequence of various pathophysiological mechanisms (ischemic demyelination, gliosis, astrocytic proliferation and inflammation),[35,36] which could result from vascular insults, or neuronal injury; or both [18,38,40]

A recent case-control study reported an association of plasma ADMA levels with presence of cerebral small vessel disease (SVD), which was defined by the presence of lacunar stroke and/or leukoaraiosis on brain CT or MRI.[14] The investigators found that higher plasma ADMA levels were associated with leukoaraiosis severity, but not with an increased prevalence of clinically evident lacunar strokes. However, silent as opposed to all brain infarcts were not specifically assessed as an outcome in this study. Conversely, in our study whereas we did

observe an association of ADMA with SBI, this was not true for the risk of prevalent WMH. Our results for WMH may differ from those of Khan et al., perhaps our study participants had a lower burden of WMH and vascular risk factors, and lower mean ADMA levels (0.54 versus 0.81μmol/l in the prior study). The observed differences could also be related to the different MRI techniques used to assess volume of WMH in the two studies; we used a quantitative method, whereas Khan et al. use a qualitative technique.[14] Interestingly, other novel risk factors, such as elevated plasma tHcy levels, have also shown an association with prevalent SCI, but not with WMH in the Framingham study sample.[20]

From a clinical perspective, elevated plasma ADMA concentrations may partly explain the elevated risk of incident TIA and stroke that has been associated with silent brain infarcts.[34] Several studies have reported an association between elevated plasma ADMA levels and an increased risk for clinical events,[12,13] although a large population-based study in patients with coronary artery disease found that ADMA was an independent marker predicting the risk of future total cardiovascular events and death, but not the risk of clinical stroke.[37] Perhaps, in that study there were too few cerebrovascular events (n=45) to detect a true association with clinical stroke. In a recent report from the Population Study of Women in Gothenburg, baseline ADMA levels were associated with an approximately 30% increase in risk of CVD events (MI and stroke) over a 24 year follow-up period in women.[38] In our Framingham Offspring sample we have had too few clinical stroke and TIA events to permit an assessment of the association between plasma ADMA levels and clinical stroke.

The strengths of our study are the use of a community-based sample under rigorous surveillance for clinical stroke and TIA, the relatively younger age of our sample, the validated volumetric MRI techniques and the recording of MRI measures by radiologists blinded to ADMA levels. In sequential models, we were also able to examine the association after adjustment for concurrent vascular risk factors, use of statin and antithrombotic therapies and for serum creatinine and plasma tHcy levels. The effect of ADMA on risk of SBI was independent of these traditional stroke risk factors. Further studies of this pathway could clarify if ADMA mediates the observed association of plasma tHcy with SBI.[20]

A limitation of our study is the predominantly white ethnicity of the Framingham Offspring sample that could limit the generalizability of our findings to other ethnicities. We have only single occasion measurements of both plasma ADMA and brain MRI, and could not evaluate the impact of changes in plasma ADMA on MRI phenotypes, or assess the relation between baseline plasma ADMA levels and the risk of incident SCI or longitudinal changes in WMH; moreover we do not know if the observed MRI changes preceded or followed the baseline examination wherein ADMA levels were assessed. Thus, our cross-sectional analysis does not permit us to confirm or refute a causal role for ADMA.

Additional molecular and experimental studies are required to assess the role of ADMA in brain injury. Nevertheless, our findings raise the possibility that elevated ADMA levels may be a biomarker of subclinical brain injury, or even represent a target for therapeutic interventions. Further prospective studies will clarify if baseline ADMA levels have an additive value in stroke risk stratification when combined with other traditional and novel biomarkers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Age-Specific Quartiles of Plasma ADMA Levels in Framingham Offspring

Age	N	Q1	Q2	Q3	Q4
<40	49	0.18–0.45	0.45–0.51	0.52–0.56	0.57–0.78
40–49	363	0.19–0.44	0.44–0.50	0.50–0.59	0.60–1.02
50–59	692	0.20–0.45	0.45–0.52	0.52–0.60	0.60–1.08
60–69	548	0.21–0.47	0.47–0.55	0.55–0.63	0.63–1.01
70+	253	0.27–0.49	0.49–0.56	0.57–0.65	0.65–1.10

Plasma ADMA units: μmol/l

Table 2

Baseline characteristics of Study Sample

Stroke Risk Factors/Other Covariates	**n=2013**
Women, %	53
Age, yrs	58±9.5
Systolic Blood Pressure, mm Hg	126±18
Anti-hypertensive medication, %	24%
Current smokers, %	14%
Diabetes mellitus, %	9.5%
Prevalent cardiovascular disease, %	7.5%
Atrial fibrillation, %	1.85%
Log (plasma homocysteine)	2.21±0.29
Serum creatinine, μmol/L	101.7±16.3
Outcome Variables	
Silent Brain Infarct (SBI)	10.7%
Large White Matter Hyperintensity Volume (LWMH)	12.6%

Values are mean±SD for continuous variables.

Table 3

Association of Plasma ADMA and brain MRI variables

	Large WMH		Silent Brain Infarct	
Model	**Odds Ratio (95% CI)**	**p**	**Odds Ratio (95% CI)**	**p**
Model 1[*]				
1 SD ↑	1.00 [0.87–1.14	1.00	**1.15 [1.00–1.32]**	**0.04**
Q1		Ref		Ref
Q2	0.98 [0.67–1.43]	0.90	**1.54 [1.02–2.34]**	**0.04**
Q3	1.13 [0.78–1.63]	0.52	1.34 [0.88–2.05]	0.17
Q4	0.96 [0.66–1.40]	0.82	1.49 [0.98–2.26]	0.07
Trend across quartiles		0.98		0.06
Q234 vs. Q1	1.02 [0.75–1.39]	0.90	**1.46 [1.02–2.07]**	**0.04**
Model 2[†]				
1 SD ↑	1.00 [0.87–1.14]	0.95	**1.16 [1.01–1.33]**	**0.04**
Q1		Ref		Ref
Q2	0.98 [0.67–1.44]	0.92	**1.56 [1.03–2.37]**	**0.04**
Q3	1.09 [0.76–1.59]	0.63	1.26 [0.82–1.94]	0.28
Q4	0.95 [0.65–1.39]	0.80	1.49 [0.98–2.27]	0.06
Trend across quartiles		0.95		0.16
Q234 vs. Q1	1.01 [0.74–1.37]	0.95	**1.43 [1.00–2.04]**	**0.05**
Model 3[‡]				
1 SD ↑	1.00 [0.87–1.14]	0.97	**1.15 [1.00–1.33]**	**0.05**
Q1		Ref		Ref
Q2	0.96 [0.66–1.41]	0.84	**1.57 [1.03–2.39]**	**0.04**
Q3	1.06[0.73–1.54]	0.76	1.24 [0.81–1.91]	0.33
Q4	0.95 [0.65–1.39]	0.81	1.48 [0.97–2.26]	0.07
Trend across quartiles		0.94		0.18
Q234 vs. Q1	0.99 [0.73–1.35]	0.95	**1.43 [1.00–2.03]**	**0.05**

[*] Model 1 (adjusted for age, sex and time to MRI)

[†] Model 2 (Model 1 plus additionally adjusted for atrial fibrillation, history of cardiovascular disease, smoking, diabetes mellitus, systolic blood pressure, anti-hypertension therapy)

[‡] Model 3 (Model 2 plus additionally adjusted for serum creatinine)