Diabetic retinopathy is associated with elevated serum asymmetric and symmetric dimethylarginines

Short title: Serum dimethylarginines and diabetic retinopathy

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Objective: Asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-arginine directly influence nitric oxide production. Our objective was to test whether serum ADMA, SDMA or L-arginine levels correlate with diabetic retinopathy (DR) subtype or severity.

Methods: 162 subjects with type 1 diabetes and 343 with type 2 diabetes, of which 329 subjects had no DR, 27 non-proliferative DR (NPDR), 101 proliferative DR (PDR) and 107 clinically significant macular edema (CSME) were recruited. Blinding DR was defined as severe NPDR, PDR or CSME. Serum ADMA, SDMA and L-arginine concentration was determined by mass spectroscopy.

Results: In multivariate analysis, blinding DR, PDR and nephropathy were associated with significantly increased serum levels of ADMA(p<0.001), SDMA(p<0.001) and L-arginine(p=0.001). Elevated ADMA(p<0.001) and SDMA(p<0.001) were also significantly associated with CSME.

Conclusion: Severe forms of DR are associated with elevated serum ADMA, SDMA and L-arginine. Further investigation is required to determine whether these findings are of clinical relevance.
Endothelial dysfunction and impaired ocular hemodynamics underlying diabetic retinopathy (DR) development is associated with decreased nitric oxide (NO) synthase activity and NO bioavailability, resulting in vasoconstriction and increased reactive oxygen species (1). Serum asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA) and L-arginine are involved in the NO pathway, directly influencing NO production. This study investigated the association between diabetic retinopathy subtypes, and serum levels of ADMA, SDMA and L-arginine in an Australian cohort of 505 subjects with type 1 or type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects were recruited from ophthalmology and endocrinology outpatient clinics of three tertiary hospitals in Adelaide, South Australia. Ethics approval was obtained from the relevant Human Research Ethics Committees. The cohort consisted of 162 subjects with type 1 and 343 with type 2 diabetes. Retinopathy status for the worst eye was graded according to the Early Treatment and Diabetic Retinopathy Study criteria (2). If either eye had CSME irrespective of other DR gradings, the patient was also classified as having CSME. Blinding retinopathy was defined as severe non-proliferative diabetic retinopathy (NPDR), or proliferative diabetic retinopathy (PDR), or clinically significant macular edema (CSME).

Blood pressure (BP) and body mass index (BMI) were measured. Renal function tests (serum creatinine, urine albumin and albumin:creatinine ratio), serum cholesterol and HbA1c levels (mean of 3 recent levels) were obtained. Patients were classified as hypertensive if they were on antihypertensive medication, or had BP greater than or equal to 140/90 mmHg at recruitment. Hypercholesterolemia was defined as total cholesterol of greater than 5.5 mmol/L, or current use of lipid lowering medication. Nephropathy was defined as urine albumin ≥30mg/day.

Serum concentrations of arginine and its di-methylated metabolites ADMA and SDMA were determined by liquid chromatography-tandem mass spectrometry of the butyl esters (3) on an Applied Biosystems 3200 Q-Trap instrument (Applied Biosystems, Scoresby, Victoria).

Statistical analyses were undertaken in SPSS (v15.0 SPSS Inc, Chicago, IL). A p-value of less than 0.05 was considered significant. Baseline clinical characteristics of cases and controls were compared using the t-test or chi-square tests as appropriate. Serum ADMA, SDMA and L-arginine concentrations were log transformed and association with DR assessed by a hierarchical multiple regression procedure for multivariate analysis.

RESULTS

Of 505 participants, 330 subjects had no DR (105 type 1 and 225 type 2 diabetes), and 175 were classified as having blinding DR (57 type 1 and 118 type 2 diabetes). In the blinding DR group, 27 had severe NPDR (4 type 1 and 23 type 2 diabetes), 101 PDR (42 type 1 and 59 type 2 diabetes) and 108 CSME (26 type 1 and 82 type 2 diabetes).

Disease duration, sex, age, hypertension, hypercholesterolemia, nephropathy and BMI were significantly correlated with DR (p<0.05). Blinding DR (Figure 1) and PDR were strongly associated with elevated serum ADMA (p<0.001), SDMA (p<0.001) and L-arginine (p=0.001) after adjustment for associated covariates. In type 1 diabetes, blinding DR was associated with significantly increased ADMA (p<0.001) and SDMA (p<0.001). In patients with type 1 diabetes and PDR there was a strong association with ADMA (p<0.001) and
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SDMA (p<0.001), and a borderline association with L-arginine (p=0.04). In type 2 diabetes, both blinding DR and PDR were significantly associated with elevated ADMA and SDMA (p=0.013 and p<0.001 respectively for blinding DR and p=0.014 and p<0.001 respectively for PDR). CSME was significantly associated with elevated ADMA (p<0.001) and SDMA (p<0.001) when both types of diabetes were combined. However, in type 1 diabetes alone only SDMA showed a significant elevation (p<0.001) and no significant association of the analytes with CSME in type 2 diabetes was found.

Age, disease duration, hypertension, BMI, hypercholesterolemia, smoking and DR were found to be significantly correlated with nephropathy (p<0.05). Nephropathy was associated with ADMA (p<0.001), SDMA (p<0.001) and L-arginine (p=0.001) after adjustment for associated covariates. All three analytes were associated with nephropathy in type 1 diabetes (ADMA p<0.001 and SDMA p<0.001, L-arginine p=0.034). However, only ADMA (p=0.03) and SDMA (p<0.001) were associated with nephropathy in type 2 diabetes.

The mean levels of all three analytes in participants with blinding DR but nephropathy subjects excluded (n=110) were compared to the mean levels in nephropathy but blinding DR excluded (n=68) and no significant differences were found (p>0.5).

CONCLUSIONS

ADMA, SDMA and L-arginine are involved in the production of NO, a key player in both microvascular damage pathogenesis and DR(1). We found that all three are significantly elevated in patients with blinding DR and PDR, irrespective of diabetes type. This study is the first to report an association between elevated levels of ADMA and SDMA with CSME.

Four previous studies investigated serum ADMA levels in DR(4-7). Three reported elevation of ADMA in DR participants(4-6). Only Malecki et al assessed the association of both SDMA and L-arginine with DR in type 2 diabetes, finding an association of SDMA with DR(5). Tarnow et al(7) found ADMA levels were not significantly increased in any form of DR in 600 subjects with type 1 diabetes. Our study was deliberately enriched with subjects with blinding DR so differences in DR phenotype affecting study power may be factors in the comparison.

The effect of nephropathy on DR(8; 9) could potentially be mediated by elevated dimethylarginines, as all 3 analytes are renally cleared and ADMA and SDMA are elevated by reduced renal clearance(7; 10). We observed a significant association of all three analytes with nephropathy. Serum SDMA in patients with nephropathy, especially end-stage, are known to be markedly higher than ADMA(10; 11). Similarly, we found higher SDMA compared to ADMA in participants with nephropathy in addition to retinopathy. One possibility is that decreased renal clearance of these analytes may lead to elevated serum concentrations directly impacting on DR development. Other factors which could influence ADMA include hyperglycemia induced inhibition of dimethylarginine dimethylaminohydrolase which degrades ADMA(12), the effects of insulin resistance(13) or medications including oral hypoglycaemic agents(13; 14) and angiotensin converting enzyme inhibitors(15).

Further prospective and functional studies are required to investigate the clinical and pathological significance of elevated ADMA, SDMA and L-arginine in DR development, and the relationship to nephropathy.

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References
Figure 1: Boxplots of untransformed concentrations of L-arginine, ADMA and SDMA (µmol/L) in all subjects with no DR (n=330) and blinding DR (n=175) are shown, regardless of type of diabetes. Data are shown as the 25, 50 and 75 percentiles (represented by grey boxes), range (shown as whiskers, outliers have been removed) and the median (white horizontal line). Details of the mean, standard deviation and adjusted p values for each analyte are provided under the corresponding boxplot.

Note: p values have been adjusted for type of diabetes, diabetes duration, age, HTN, hypercholesterolemia and nephropathy.