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Serum Asymmetric Dimethy L- Arginine Level and its Relation to Different Variables among Type 1 Diabetic Children

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Abstract: Patients with type 1 diabetes mellitus (DM) are very susceptible to vascular complications. Endothelial dysfunction is the earliest feature for the vascular complications of DM. Asymmetric N^G, N^G-dimethyl-l-arginine (ADMA) is a sensitive indicator for endothelial dysfunction and thus of vascular complications of DM. The aim of this study was to investigate the circulating ADMA level and its relation to metabolic parameters especially HbA1c in type 1 diabetic children. The current study was conducted on 30 children with type 1 DM (cases) of the outpatient endocrine Clinic in Cairo University Pediatric Hospital and 20 healthy children (controls) whose age and gender were similar to those of cases. Serum ADMA level was significantly increased in cases compared with that of controls. There was excellent positive correlation between ADMA levels and glycosylated heamoglobin (HbA1c) and diabetes duration among cases. In conclusion, increased circulating ADMA levels have been demonstrated in type 1 diabetic children who do not suffer from diabetic vascular complications. Monitoring ADMA levels in diabetic patients is of utmost importance to predict and then prevent the development of cardiovascular complications.

Key words: ADMA, Type I diabetes children, HbA1c, Vascular complications.

INTRODUCTION

Nitric oxide (NO), synthesized from the amino acid precursor l-arginine by NO synthase (NOS), is a major endothelial-derived vasoactive mediator which is involved in the maintenance of vascular homeostasis (Vallance and Chan**,** 2001). There are two types of endogenous NOS inhibitors in circulation, NG-monomethyl-l-arginine and asymmetric N^G, N^G-dimethyl-l-arginine (ADMA) (Boger, 2003). ADMA is the major inhibitor of NOS by competing with l-arginine and metabolized by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) (Boger, 2004). ADMA is eliminated from the body by renal excretion so accumulation of ADMA was first shown in patients with chronic renal failure (Vallance et al., 1992). Elevated plasma ADMA levels have also been reported in hypercholesterolemia (Boger et al., 1998), hypertension (Perticone et al., 2005), and insulin resistance syndrome (Chan and Chan, 2002). Besides, there is substantial evidence that high circulating ADMA levels are associated with endothelial dysfunction and increased risk of atherosclerosis (Boger, 2003). A previous prospective study has revealed that plasma ADMA concentrations act as an independent risk factor for cardiovascular mortality in patients with diabetes mellitus disease (Zoccali et al., 2001).

Endothelial dysfunction is the earliest feature for the vascular complications of diabetes mellitus (DM) and its underlying mechanisms are not fully established (Schalkwijk and Stehouwer, 2005). Changes in NOS pathway associated with endothelial dysfunction have an important role in the course of type 1 DM (Chan et al., 2000). Because ADMA is an endogenous competitive inhibitor of NOS, elevated ADMA levels may contribute to the impaired NOS pathway in patients with type 1 DM. There are few data regarding the association between elevated plasma ADMA levels and type 1 DM.

Glycated hemoglobin (hemoglobin A1c, HbA1c, A1C, or Hb1c; sometimes also HbA1c) is a form of hemoglobin that is measured primarily to identify the average plasma glucose concentration over prolonged periods of time. It is formed in a non-enzymatic glycation pathway by hemoglobin's exposure to plasma glucose. Normal levels of glucose produce a normal amount of glycated hemoglobin. As the average amount of plasma glucose increases, the fraction of glycated hemoglobin increases in a predictable way. This serves as a marker for average blood glucose levels over the previous months prior to the measurement (Murugan et al., 2010). The 2010 American Diabetes Association Standards of Medical Care in Diabetes added the HbA1c $\geq$ 48 mmol/mol ($\geq$6.5%) as a criterion for the diagnosis of diabetes (Diabetes Care, 2010).

In diabetes mellitus, higher amounts of glycated hemoglobin, indicating poorer control of blood glucose levels, have been associated with cardiovascular disease, nephropathy and retinopathy. Monitoring HbA1c in type-1 diabetic patients may improve treatment (Larsen et al., 1990).

The aim of the present study was to investigate the circulating ADMA level and its relation to metabolic parameters especially HbA1c in type 1 diabetic children without vascular complications.

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Subjects and Methods:

Thirty children with type 1 DM (cases) and twenty healthy children of age and sex matched (controls) were enrolled in the present study. Mean ages were 9.4 ± 2.2 (7–14yr) for cases. Some characteristics of the cases and controls are presented in Table (1). Diagnosis of type 1 DM was based on the American Diabetes Association criteria (Expert Committee on the Diagnosis and classification of Diabetes Mellitus, 2002). Patients did not have hepatic or renal dysfunction, chronic inflammatory or clinically significant infectious diseases. None of the patients were taking any medication other than insulin. ADMA, total cholesterol and triglycerides (Tg) were measured in all subjects. Glycated hemoglobin (HbA1c) was measured in cases.

Venous blood samples were drawn after a 12-h overnight fast. Samples were separated and stored at −80° C until analysis. Serum asymmetric dimethyl arginine (ADMA) was determined by using enzyme linked immunosorbent assay (ELISA) kit provided from Immunodiagnostic AG, Germany, according to the method described by Nijveldt et al. (2003). Serum insulin was analyzed by mean of an enzyme amplified chemiluminescence assay (IMMULITE, Diagnostic Products Corp., Los Angeles, according to the method of Easeham, (1985). Serum levels of total cholesterol were determined colorimetrically using a Stanbiolaboratory kit (USA) according to the method of Richmond, (1973). Serum level of triglyceride was determined by colorimetric method using Bio-diagnostic Kit (Egypt) according to the method described by Fassati and Prencipe (1982). Glycated hemoglobin (HbA1c) was measured in whole blood chromatographically and colorimetrically using a kit obtained from BioSystems (Spain) according to the method of Roberts et al., (2002).

The study was approved by the ethical committee of the National Research Centre. All subjects gave informed consent to participation.

Statistical Analysis:

Data management and analysis were performed using Statistical Package for Social Sciences (SPSS). Comparisons between cases and controls with respect to numeric variables were done using the Student t-test. To measure the strength of the association between numerical data Pearson's correlation coefficients were calculated (Dawson and Trapp, 2001). P-values < 0.05 were considered significant.

Results:

As shown in Table (1), the mean age of cases was 9.4±2.2 years, and the mean duration of diabetes among cases was 4.23 ±2.07 years. The mean serum ADMA concentration (1.1±0.6 µmol/L) showed significant increase in cases compared with that of controls (0.3±0.3 µmol/L) (P<0.001).The mean HbA1c in cases was 8.2±0.9%. Serum insulin level revealed significant increase (P<0.001) in cases (27.3±6.6 IU/ml) versus control value (7.8±3.0 IU/ml). The mean total cholesterol level was 174.6±11.4 mg/dl and Tg was 99.3±13.0 mg/dl in cases which indicate significant increase in both lipid parameters compared with those in the controls (162.2±11.0 mg/dl) and (83.0±7.0mg/dl) respectively (P<0.001).

Table 1: Characteristics of patients and controls.

	Groups								
	Cases				Controls				
Variables	Mean	±	Std. Deviation	Standard error	Mean	±	Std. Deviation	Standard error	P-value
Age (years)	9.4	±	2.2		9.5 ± 2.4				>0.05
Diabet. Duration (Years)	4.23	±	2.07	0.38					
ADMA (µmol/L)	1.1	±	0.6	0.07	0.3	±	0.3	0.11	<0.001
HbA1c (%)	8.2	±	0.9						
Insulin (IU/ml)	27.3	±	6.6	0.68	7.8	±	3.0	1.21	<0.001
Cholesterol (mg/dl)	174.6	±	11.4	2.46	162.2	±	11.0	2.09	<0.001
Tg (mg/dl)	99.3	±	13.0	1.57	83.0	±	7.0	2.37	<0.001

As shown in Table (2) and Fig (1), there was excellent positive correlation between ADMA levels and HbA1c among cases (r=0.828, P<0.001). Table (2) and Fig (2) also showed that ADMA has a very good correlation with diabetes duration (r=0.754, P<0.001). There was also good positive correlation between ADMA and insulin level (r=0.668, P<0.001) as shown in Table (2) and Fig (3). Also, the results in Table (2) showed that there is weak positive correlation between ADMA levels and total cholesterol level (r=0.390, P=0.005) and moderate positive correlation between ADMA levels and Tg level (r=0.471, P=0.001). No correlation was found between ADMA levels and age of patient group (r=0.214, P=0.257).

Table 2: Correlation between serum ADMA level and different biochemical parameters, diabetes duration and age of diabetic patients.

ADMA (µmol/l)			
Variables	R	p-value	Correlation Degree
HbA1c (%)	0.828	<0.001	Excellent correlation
Diabetes duration (years)	0.754	<0.001	Very good correlation
Insulin (IU/ml)	0.668	<0.001	Good correlation
Cholesterol (mg/dl)	0.390	0.005	Weak correlation
Tg (mg/dl)	0.471	0.001	Moderate correlation
Age (years)	0.214	0.257	No correlation

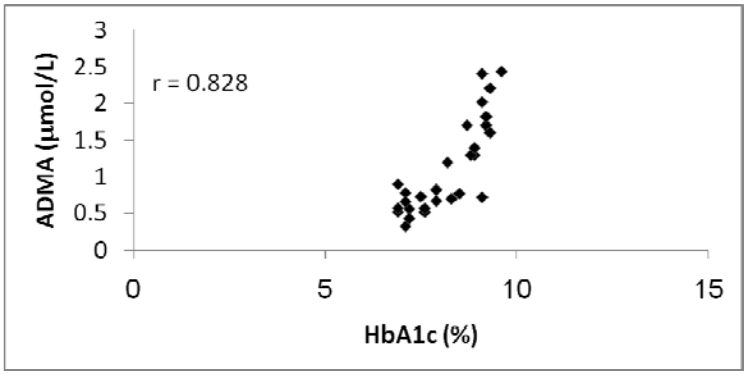

Fig. 1: Correlation between ADMA level and HbA1c in diabetic patients.

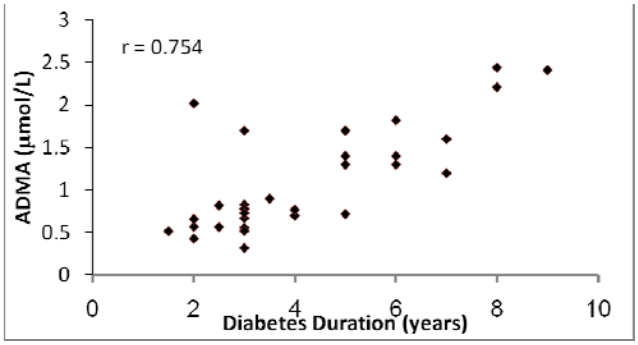

Fig. 2: Correlation between ADMA level and diabetes duration in diabetic patients.

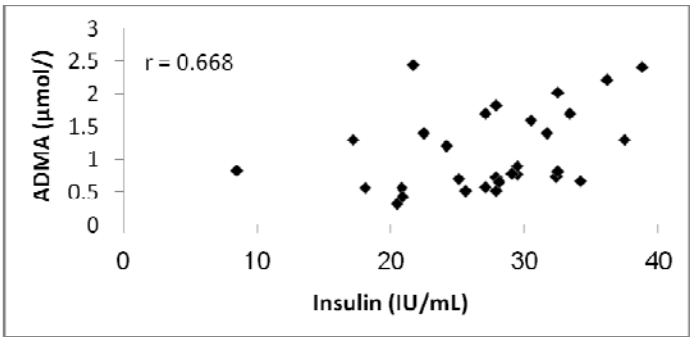

Fig. 3: Correlation between ADMA level and insulin in diabetic patients.

Discussion:

The present study showed significant increase in serum ADMA levels in type 1 diabetic patients when compared with the controls.

This is in agreement with Alev et al., (2007), who reported that circulating ADMA levels were higher in type 1 diabetic patients compared to that of control group. In another previous study (Mittermayer, 2005), in 11 patients with type 1 diabetes, higher ADMA levels was determined before the exercise when compared with controls. Mean serum ADMA concentration in our cases was 1.1 ± 0.6 µmol/L and this level is higher than the

level found in the study of Tarnow et al. (2004) (0.46 ± 0.08 µmol/L) and the study of Mittermayer et al. (2005) (0.54 ± 0.02 µmol/L) but lower than that in the study of Yasuda et al. (2006) (4.8 ± 1.5 µmol/L). These studies included patients with either type 1 or type 2 DM, which might be the reason of this difference. Also, this discrepancy may be due to the different reference methods used in estimating ADMA level. In the contrary to our study Huemer et al. (2011) found that ADMA concentrations were significantly lower in the patients with type 1DM than the controls. This contraindication may be due to that nitric oxide may exert oxidative stress by generating free radicals. In these circumstances, ADMA would protect the system from nitric oxide overproduction and perpetuation of oxidative stress. Thus, low ADMA concentrations in children with DM1 may be an indicator of impaired protection against oxidative stress. Glowinsika et al. (2010) did not find differences in ADMA level in diabetic children with the presence of additional diseases being cardiovascular risk factors.

Our finding of increased ADMA level in uncomplicated type 1 diabetic patients indicates that the endothelial dysfunction associated with increased ADMA concentrations seems to begin before the detectable vascular damage in type 1 diabetic patient. Additionally, type 1 diabetic patients had increased levels of ADMA leading to endothelial damage, even if vascuar complications do not exist. Therefore, measurement of ADMA serum levels as marker of endothelial dysfunction may provide an opportunity for the prevention of irreversible endothelial damage in these patients.

As regard to the cholesterol and Tg levels, our study showed the increased serum levels of both lipid parameters in cases as compared to the controls. This is in controversy to the study performed by Jehlika et al. (2009), who found insignificant difference between the two groups concerning cholesterol and Tg serum levels. This may be due to that the duration of diabetes is different between our patients and the patients of that study.

In the present study, we found an excellent positive correlation between ADMA level and HbA1c among cases. The increase in ADMA concentrations in hyperglycemic media may be associated with the enzyme arginine methyltransferase, which synthesizes ADMA, because hyperglycemia-induced oxidative stress up-regulates the expression of arginine methyltransferases (Maas, 2005). In the contrary Marcovecchio et al. (2011) and Huemer et al. (2011)**,** found a significant negative association between HbA1c and ADMA levels in diabetic patients but Alev et al. (2007) stated that no correlation was found between ADMA level and HbA1c in diabetic patients.

In the current study, we reported a significant very good positive correlation between ADMA levels and diabetes duration among cases. This is in disagreement with Alev et al. (2007), who did not find any significant correlation between ADMA levels and diabetes duration.

Conclusion:

In conclusion, the increased ADMA concentrations have been demonstrated in type 1 diabetic patients who do not suffer from diabetic vascular complications. Moreover, there is positive association between ADMA levels and both HbA1c and diabetes duration. Thus, we could suggest that chronic hyperglycemia might up-regulate mechanisms implicated in ADMA production. Monitoring of ADMA levels in diabetic patients is of utmost importance to predict and then prevent the development of cardiovascular complications.

REFERENCES

Altinova, A.E., M. Arslan, A. Sepici-Dincel, M. Akturk, N. Altan and F.B. Toruner, 2007. Uncomplicated type 1 diabetes is associated with increased asymmetric dimethylarginine concentrations. J. Clinical Endocrinology., 92: 1881-1885.

Boger, R.H., 2003. The emerging role of ADMA as a novel cardiovascular risk factor. Cardiovasc Res., 59: 824-833.

Boger, R.H., 2004. Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor. J. Nutr., 134: 2842S-2847S.

Boger, R.H., S.M. Bode-Boger, A. Szuba, P.S. Tsao, J.R. Chan, O. Tangphao, T.F. Blaschke and J.P. Cooke, 1998. Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. Circulation., 98: 1842-1847.

Chan, N.N. and J.C. Chan, 2002. Asymmetric dimethylarginine (ADMA): a potential link between endothelial dysfunction and cardiovascular diseases in insulin resistance syndrome? Diabetologia., 45: 1609-1616.

Chan, N.N., P. Vallance and H.M. Colhoun, 2000. Nitric oxide and vascular responses in type I diabetes. Diabetologia, 43: 137-147.

Dawson, B. and R. Trapp, 2001. Basic and clinical biostatistics. New York Lange Medical Boobs- Mc Graw Hill.

American Diabetes Association, 2010. Standards of medical care in diabetes. Diabetes Care., 33: 11-61.

Eastham, R.D., 1985. Biochemical values in clinical medicine 7th ED. Bristol. England. John Wrights., Sons, ITD.

Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2002. American Diabetes Association : clinical practice recommendation. Diabetes Care, 25(Suppl 1): 1-147.

Fassati, P. and L. Prencipe, 1982. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin. Chem., 28: 2077-2080.

Głowińska-Olszewska, B., W. Luczyński, J. Jabłońska, A. Otocka, B. Florys and A. Bossowski, 2010. Asymmetric dimethylarginine (ADMA) in children with diabetes type 1. Pediatr Endocrinol Diabetes Metab., 16: 137-341.

Huemer, M., B. Simma, D. Mayr, A. Mühl, E. Schober, H. Ulmer, U. Zanier and O.A. Bodamer, 2011. Low levels of asymmetric dimethylarginine in children with diabetes mellitus type I compared with healthy children. J. Pediatr., 158(4): 602-606.

Jehlika, P., F. Stozicky, J.R. Mayer, J. Varvarovska, J. Racek, L. Trefil and K. Siala, 2009. Asymmetric dimethylearginine and the effect of folate substitution in children with familiar hypercholesterolemia and diabetes mellitus type 1. Physiol. Res., 58: 179-184.

Larsen, M.L., M. Hørder and E.F. Mogensen, 1990. Effect of long-term monitoring of glycosylated hemoglobin levels in insulin-dependent diabetes mellitus. N. Engl. J. Med., 323: 1021-1025.

Maas, R., 2005. Pharmacotherapies and their influence on asymmetric dimethylargine (ADMA). Vasc. Med., 10(Suppl 1): 49-57.

Marcovecchio, M.L., B. Widmer, C. Turner, D.B. Dunger and R.N. Dalton, 2011. Asymmetric dimethylarginine in young people with Type 1 diabetes: a paradoxical association with HbA(1c). Diabet. Med., 28(6): 685-691.

Mittermayer, F., J. Pleiner, K. Krzyzanowska, G.F. Wiesinger, M. Francesconi and M. Wolzt, 2005. Regular physical exercise normalizes elevated asymmetrical dimethylarginine concentrations in patients with type 1 diabetes mellitus. Wien Klin Wochenschr, 117: 816-820.

Murugan, K., D.K. Shrivastava, S.K.B. Patil, L. Sweety, G. Debapriya, A. Bharti and L.S. Kumar, 2010. Biochemical investigation of glycosylated haemoglobin in diabetes associated nephropathy in Chhattisgarh population. Advances in Applied Science Research, 1: 106-113.

Nijveldt, R.J., T. Teerlink, B. Van der Hoven, M.P. Siroen, D.J. Kuik, J.A. Rauwerda and P.A. van Leeuwen, 2003. Asymmetrical dimethylarginine (ADMA) in critically ill patients: high plasma ADMA concentration is an independent risk factor of ICU mortality. Clin. Nutr., 22: 23-30.

Perticone, F., A. Sciacqua, R. Maio, M. Perticone, R. Maas, R.H. Boger, G. Tripepi, G. Sesti and C. Zoccali, 2005. Asymmetric dimethylarginine, l-arginine, and endothelial dysfunction in essential hypertension. J. Am. Coll. Cardiol., 46: 518-523.

Richmond, W., 1973. Preparation and properties of a cholesterol oxidase from Nocrdia spand its application to the enzymatic assay of cholesterol in serum. Clin. Chem., 19: 1350-1356.

Roberts, W.L., B.K. De, D. Brown, C.M. Hanbury, J.D. Hoyer, W.G. John, T.L. Lambert, R.B. Lundell, C. Rohlfing and R.R. Little, 2002. Effects of hemoglobin C and S traits on eight glycohemoglobin methods. Hanbury Clin. Chem., 48: 383-385.

Schalkwijk, C.G. and C.D. Stehouwer, 2005. Vascular complications in diabetes mellitus: the role of endothelial dysfunction. Clin. Sci. (Lond), 109: 143-159.

Tarnow, L., P. Hovind, T. Teerlink, C.D. Stehouwer, H.H. Parving, 2004. Elevated plasma asymmetric dimethylarginine as a marker of cardiovascular morbidity in early diabetic nephropathy in type 1 diabetes. Diabetes Care, 27: 765-769.

Vallance, P., N. Chan, 2001. Endothelial function and nitric oxide: clinical relevance. Heart, 85: 342-350.

Vallance, P., A. Leone, A. Calver, J. Collier, S. Moncada, 1992. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. Lancet, 339: 572-57.

Yasuda, S., S. Miyazaki, M. Kanda, Y. Goto, M. Suzuki, Y. Harano, H. Nonogi, 2006. Intensive treatment of risk factors in patients with type-2 diabetes mellitus is associated with improvement of endothelial function coupled with a reduction in the levels of plasma asymmetric dimethylarginine and endogenous inhibitor of nitric oxide synthase. Eur Heart J., 27: 1159-1165.

Zoccali, C., S.M. Bode-Boger, F. Mallamaci, F.A. Benedetto, G. Tripepi, L. Malatino, A. Cataliotti, I. Bellanuova, I. Fermo, J.C. Frolich, R. Boger, 2001. Asymmetric dimethylarginine (ADMA): an endogenous inhibitor of nitric oxide synthase predicts mortality in end-stage renal disease (ESRD). Lancet, 358: 2113-2117.