

SERUM ASYMMETRIC DIMETHYL ARGININE (ADMA) AND ARGININE LEVELS AS PREDICTORS OF VASCULAR COMPLICATIONS IN TYPE 2 DMKarthik G.^{1*} and Ravi Kumar Y. S.²¹Post Graduate in General Medicine, JSS Medical College Mysuru, JSSAHER, Mysuru, Karnataka India – 570004.²Professor in General Medicine, JSS Medical College Mysuru, JSSAHER, Mysuru, Karnataka India – 570004.***Corresponding Author: Dr. Karthik G.**

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ABSTRACT

Insulin resistance in DM is associated with reduced nitric oxide (NO) bioavailability and endothelial dysfunction.^[1] Endothelial dysfunction due to reduced nitric oxide (NO) bioavailability is an earliest change and prominent feature of insulin resistance.^[1,5,6,7] Deficiency of NO increases vascular resistance and promotes atherogenesis. L-Arginine is a common precursor for nitric oxide (NO), act as substrate for enzymes involved in nitric oxide synthases.^[2,3] Asymmetric di methyl arginine (ADMA) which is a product of protein methylation on arginine is an endogenous competitive inhibitor of NO synthesis that exerts pro atherogenic effect. This study aim to correlate levels of serum Arginine and serum ADMA in DM with vascular complications and without vascular complications as compared with non diabetic subjects. This study concluded that Serum Arginine concentration is low among Diabetics as compared with Non diabetics and Arginine level was significantly low in subjects with vascular complications associated with diabetes mellitus as compared with Diabetics without vascular complications and Non diabetic subjects and also Serum arginine was inversely related to HbA1c. Serum ADMA level was high among Diabetics as compared with Non Diabetics and Serum ADMA levels were significantly high among diabetes with vascular complications as compared with diabetes without vascular complications and Non diabetic subjects and Serum ADMA levels were elevated in proportion to HbA1c. Hence it is suggestive that early supplementation of Arginine which is a precursor of NO, may retard progression of vascular complications associated with Diabetes mellitus.^[8,9]

KEYWORDS: ADMA: Asymmetric di methyl arginine, DM: Diabetes Mellitus.**INTRODUCTION**

Diabetes is a metabolic disease due to hyperglycemia resulting from either impaired insulin production, its action or both. It is associated with numerous complications and early detection and prevention is a key factor in management of DM. Diabetes is associated with vascular complications which may be micro vascular (Diabetic retinopathy, Diabetic neuropathy, Diabetic nephropathy) or macro vascular complications (Diabetic foot disease, coronary artery diseases and cerebrovascular accidents). Endothelial dysfunction is the earliest change that is associated with vascular complications of DM, NO (Nitric oxide) plays a major role in vasodilatation as it is a potent vasodilator which is released by the endothelium.^[4] Serum arginine being precursor of NO was found to be low in various studies conducted on diabetic population, ADMA which is a methylation product of arginine and an endogenous inhibitor of arginine was high in diabetic population, that results in impaired NO production and vascular complications associated with diabetes mellitus, hence early supplementation of arginine in diabetics may delay vascular complications associated with diabetes.

MATERIALS AND METHODS

It was a cross sectional descriptive study of patients admitted in the Department of General Medicine at JSS Hospital, Mysuru. All the study subjects were enrolled into the proforma with all the relevant clinical details including therapeutic details and other associated comorbidities.

- Patients satisfying inclusion criteria were evaluated for DM using ADA criteria, and were further evaluated for vascular complications associated with diabetes mellitus and classified into 3 groups i.e Group A –Diabetes with micro or macro vascular complications, Group B – Diabetes without micro or macro vascular complications and Group C – Non Diabetic subjects. Individuals with type 1 DM, history of unstable angina, Acute coronary artery disease, recent cardiovascular intervention within last 6 months, hypertension, Significant liver dysfunction i.e AST, ALT levels higher than 2.5 times upper limit of reference normal range). Renal dysfunction i.e serum creatinine more > 1.5mg history of alcohol consumption, thyroid diseases or on drugs like oral steroid were excluded from study.

Serum arginine and ADMA levels were estimated and correlated among these groups. 120 subjects were included in the study and out of which 80 were diabetic and were compared with 40 subjects without diabetes mellitus.

All the subjects were evaluated biochemically for fasting blood glucose, post prandial blood glucose, fasting lipid profile and glycated Hb (HbA1c) to confirm DM according to ADA diagnostic criteria.

Table 1: Diagnostic criteria according to ADA.

Inference	HbA1C
Normal	less than 5.7%
Prediabetes	5.7% to 6.4%
Diabetes	6.5% or higher
Inference	Fasting Plasma Glucose (FPG)
Normal	less than 100 mg/dl
Prediabetes	100 mg/dl to 125 mg/dl
Diabetes	126 mg/dl or higher
Inference	Oral Glucose Tolerance Test (OGTT)
Normal	less than 140 mg/dl
Prediabetes	140 mg/dl to 199 mg/dl
Diabetes	200 mg/dl or higher

Study subjects were further evaluated for complications related with diabetes mellitus, Evaluation for retinopathy was done by ophthalmologist and findings were graded as mild moderate and severe NPDR or PDR. Retinopathy status for the worst eye was graded. Peripheral

neuropathy was tested using 10 g monofilament, vibration perception was assessed using 128 hz tuning fork, and subjective sensory symptoms described by patients like paresthesia or dysesthesia were also taken in to account. Nephropathy was evaluated using spot urine micro albumin and creatinine ratio. Nephropathy was defined as urinary albumin and creatinine ratio more than 30 mg/g. UAC less than 30mg/g was considered normal and value between 30-300 mg/g was considered as micro albuminuria and classified as macro albuminuria when UACR more than 300 mg/g. Fasting lipid profile was done and was considered abnormal if Triglycerides were more than or equal to 150 mg/dl, HDL less than or equal to 40mg/dl and if LDL more than or equal to 140 mg/dl. PVD was evaluated using doppler study of lower limb and ABPI (Ankle brachial pressure index) where 0.9-1.3 was considered normal and less than 0.9 as abnormal. ECG and Echocardiographic assessment was done to look for IHD and past history of IHD was considered, patients having any of these were considered as CAD. Diabetic patients for history of neurological weakness, TIA, previous cerebro vascular accidents were considered.

Mean serum ADMA and Arginine levels were assessed among diabetic with complications and without complications which were then compared with non diabetic subjects, Blood samples were collected from study subjects and was subjected to centrifugation and serum was separated and stored at -20 Celsius and was analyzed using ELSIA kits. (Sincere Biotech Co, Ttd)

RESULTS

Table 1: Comparison of Mean Arginine among study subjects in all the study groups.

	Group					
	Diabetic With Complications		Diabetic Without Complications		Non Diabetic	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Serum arginine	70.81	5.86	71.15	8.33	80.48	4.74

F = 57.622 p= 0.001

In the present study it was found that mean serum arginine levels were statistically significant among the three groups.

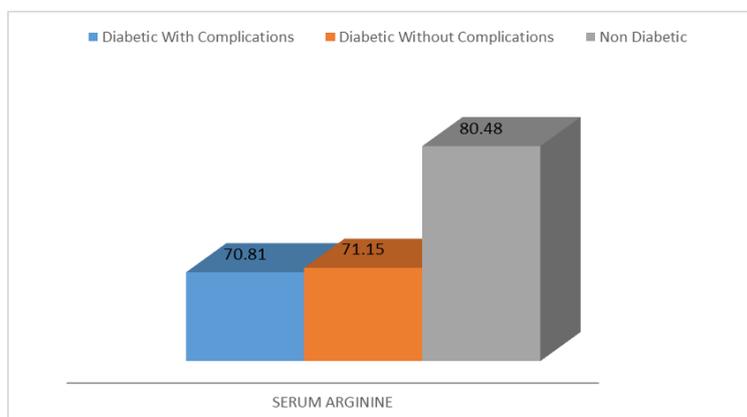


Figure 1: Graph wise Comparison of Mean Serum Arginine among study subjects in all the study groups.

Table 2: Comparison of Mean Serum ADMA among study subjects in all the study groups.

	Group					
	Diabetic With Complications		Diabetic Without Complications		Non Diabetic	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Serum adma	0.48	0.11	0.43	0.08	0.34	0.04

F = 44.910 p=0.001

In the present study it was found that mean serum ADMA levels were statistically significant among the three groups.

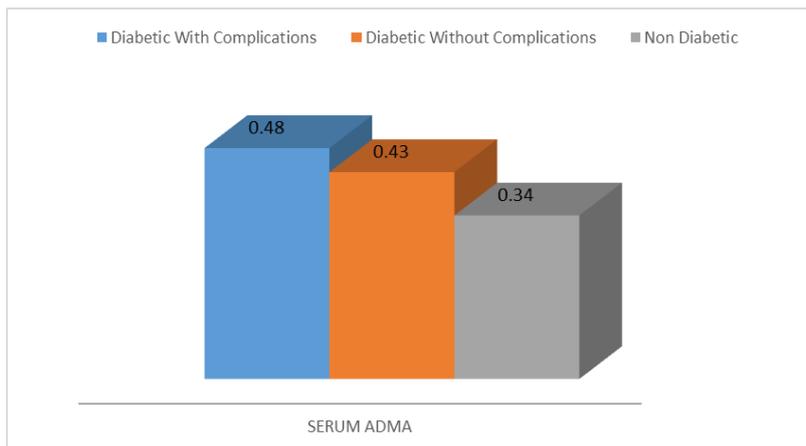


Figure 2: Graph wise Comparison of Mean Serum ADMA among study subjects in all the study groups.

Table 3: Correlation between HbA1c and Serum Arginine and Serum ADMA Levels among study subjects in all the study groups.

		Correlations		
		Hba1c	Serum arginine	Serum adma
Hba1c	Pearson correlation	1	-.516**	.615**
	Sig. (2-tailed)		.000	.000
	N	120	120	120
Serum arginine	Pearson correlation	-.516**	1	-.702**
	Sig. (2-tailed)	.000		.000
	N	120	120	120
Serum adma	Pearson correlation	.615**	-.702**	1
	Sig. (2-tailed)	.000	.000	
	N	120	120	120

** . Correlation is significant at the 0.01 level (2-tailed).

On Applying Pearson Co relation between HbA1c level with Serum Arginine and Serum ADMA levels, The correlation was found to be negative between Serum

Arginine and HbA1c and Positive Co relation was seen between HbA1c and Serum ADMA levels. Both the Co relation was found to be statistically significant

Table 4: The mean level of serum Arginine was found to be 69.24 µmol/L among subjects with retinopathy and mean serum ADMA levels was 0.50 µmol/L among subjects with retinopathy. Both Serum Arginine and Serum ADMA levels were found to be significantly associated with Retinopathy.

	Retinopathy				P value
	No		Yes		
	Mean	Standard deviation	Mean	Standard deviation	
Serum Arginine	75.64	7.89	69.24	5.38	0.001
Serum ADMA	0.39	0.08	0.50	0.12	0.001

Table 5: The mean level of serum Arginine was found to be 69.92 $\mu\text{mol/L}$ among subjects with Neuropathy and mean serum ADMA levels was 0.50 $\mu\text{mol/L}$ among subjects with Neuropathy. Both Serum Arginine and Serum ADMA levels were found to be significantly associated with Neuropathy.

	Neuropathy				P value
	NO		YES		
	Mean	Standard Deviation	Mean	Standard Deviation	
Serum Arginine	74.94	8.04	69.92	5.05	0.010
Serum ADMA	0.40	0.09	0.50	0.10	0.001

Table 6: The mean level of serum Arginine was found to be 69.98 $\mu\text{mol/L}$ among subjects with Nephropathy and mean serum ADMA levels was 0.51 $\mu\text{mol/L}$ among subjects with Nephropathy. Both Serum Arginine and Serum ADMA levels were found to be significantly associated with Nephropathy.

	Nephropathy				P Value
	NO		YES		
	Mean	Standard Deviation	Mean	Standard Deviation	
Serum Arginine	74.93	7.87	69.98	6.42	0.011
Serum ADMA	0.40	0.08	0.51	0.12	0.000

Table 7: The mean level of serum Arginine was found to be 68.85 $\mu\text{mol/L}$ among subjects with PVD and mean serum ADMA levels was 0.51 $\mu\text{mol/L}$ among subjects with PVD .Both Serum Arginine and Serum ADMA levels were found to be significantly associated with PVD.

	PVD				P value
	NO		YES		
	Mean	Standard Deviation	Mean	Standard Deviation	
Serum Arginine	75.02	7.90	68.85	5.09	0.002
Serum ADMA	0.40	0.09	0.51	0.10	0.001

The mean level of Serum Arginine was found to be 74.41 $\mu\text{mol/L}$ among subjects with CVA and mean Serum ADMA levels was 0.39 $\mu\text{mol/L}$ among subjects with CVA. Both Serum Arginine and Serum ADMA levels were found to be statistically insignificant among subjects with CVA due to smaller sample size and exclusion of subjects with comorbid illness. The mean level of Serum Arginine was found to be 70.02 $\mu\text{mol/L}$ among subjects with CAD and Serum ADMA levels was 0.53 $\mu\text{mol/L}$ among subjects with CAD. Both Serum Arginine and Serum ADMA levels were found to be statistically insignificant among subjects with CAD due to smaller sample size and exclusion of subjects with comorbid illness.

DISCUSSION

Nitric oxide plays pivotal role in metabolism of vascular endothelium. Diabetes Mellitus causes reduced levels of Arginine and high ADMA which are part of the Nitric Oxide metabolism. Reduced levels of Arginine and high ADMA are predisposing factors for the aforementioned vascular complications.

Our findings are summarized as follows

- Majority of the study subjects with vascular complications were aged above 50 years which is the most common age of presentation of suggesting that vascular complications i.e both micro and macrovascular complications were more in advancing age however Age was statistically insignificant ($P=0.079$) among the groups.
- Male and female were evenly distributed in both the diabetic groups with and without vascular complications i.e 45% were Female and 55% were Male. In Non Diabetic group 37.5% were Female and 62.5% were male. The gender was statistically insignificant ($P= 0.736$) between the groups.
- We compared mean serum Arginine levels among Diabetic subjects and non Diabetic subjects and we found mean serum arginine levels as 70.98 $\mu\text{mol/L}$ among subjects with Diabetes mellitus and 80.48 $\mu\text{mol/L}$ among Non Diabetic subjects respectively. Hyperglycemic state increases the activity of Arginase which catabolizes arginine leading to lower level of arginine among diabetics and subsequently low nitric oxide and ultimately leading to vascular complications. Insulin also agument cationic amino acid transporter-1 (CAT-1) expression, which is an amino acid transporter predominant L-arginine which is expressed in endothelial cells^[10] hence in insulin resistant conditions serum arginine reduces.
- We also compared serum ADMA levels among Diabetics and Non diabetics and mean serum ADMA in diabetics was 0.45 $\mu\text{mol/L}$ as compared with non diabetics which was 0.34 $\mu\text{mol/L}$. The most probable explanation for high ADMA is that the insulin resistance and hyperglycemia in uncontrolled DM leads to oxidative stress which in turn upregulates the expression of the protein arginine methyltransferase (PRMT) and attenuates dimethyl arginine dimethylaminohydrolyase (DDAH)

activity which metabolize ADMA.

On performing co relation test it was found that the Serum Arginine levels has negative correlation with HbA1c level. As HbA1c Level increases, the Serum Arginine Level decreases indicating that the Serum arginine Level is low among subjects with poor control of diabetes mellitus.

Study subjects who were suffering from Diabetes Mellitus with vascular complications had significantly lower levels of Arginine due to decreased production of De novo Arginine and increased Arginase activity. Study association of mean serum arginine level among the study groups were found to be statistically significant between the three groups($p= 0.001$), mean Serum Arginine was found to be 70.81 $\mu\text{mol/L}$ among subjects with complications, 71.15 $\mu\text{mol/L}$ among subjects without complication and 80.48 $\mu\text{mol/L}$ among non diabetic control group respectively. It can be concluded from study that the Serum Arginine levels were found to be low among the subjects with vascular complications when compared with groups without vascular complication and non diabetic group.

The Serum ADMA Level was found to be increased among diabetics with vascular complication when compared with diabetics without vascular complication and non diabetic subjects. Among the Diabetic subjects with complications the mean serum ADMA level was 0.48 $\mu\text{mol/L}$, among diabetic group without complication it was 0.43 $\mu\text{mol/L}$ and in non diabetic the value was 0.34 $\mu\text{mol/L}$. The association was found statistically significant ($p=0.001$) among study groups.

CONCLUSION

Nitric oxide being important for vasodilatation and serum Arginine being precursor of NO

- We conclude that serum Arginine concentration is low among Diabetics as compared with Non diabetics and on further evaluation we found that Arginine levels were significantly low in subjects with vascular complications group as compared with Diabetes without vascular complications and Non diabetic subjects and also Serum arginine was inversely related to HbA1c.
- Serum ADMA levels were high among Diabetics as compared to Non Diabetics and Serum ADMA levels were significantly high in diabetes with vascular complications group as compared with DM without vascular complications and Non diabetic subjects and Serum ADMA levels elevated in proportion to HbA1c
- Hence it is suggestive that early supplementation of Arginine which is a precursor of NO may retard progression of vascular complications associated with Diabetes mellitus.

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