 DOI: 10.18231/2394-6377.2017.0023

Asymmetric dimethyl arginine as a cardiovascular risk marker in patients with hyperthyroidism: a randomised case control study in a tertiary level health care centre

Priyanka Patra[1,*], Anitha M.[2], HL Vishwanath[3]

[1]Junior Resident, [2]Assistant Professor, [3]Professor & HOD, Dept. of Biochemistry, Bangalore Medical College & Research Institute, Bangalore

***Corresponding Author:**
Email: priyankapatra@gmail.com

Abstract

Background: Cardiovascular manifestations are frequent findings in patients with thyroid hormone disorders. Asymmetric dimethylarginine (ADMA), a inhibitor of endothelial nitric oxide synthase (eNOS), blocks nitric oxide (NO) synthesis from l-arginine. So, ADMA level can be considered as cardiovascular risk marker as it is a possible contributing factors for endothelial dysfunction in hyperthyroid patients.

Objectives: The aim of the study was to assess ADMA levels in hyperthyroidism patients free of cardiovascular risk associates and further comparison with healthy controls.

Methodology: The study took place in Victoria Hospital, attached to Bangalore Medical College and Research Institute. The study groups consisted of 30 patients with diagnosed hyperthyroidism and 30 healthy sex and age matched controls. The patients with renal failure, diabetes and severe hypertension were excluded. ADMA was estimated by double-antibody sandwich enzyme-linked immunosorbent assay (ELISA).

Results: The Mean ADMA level was higher in the hyperthyroid group (1.1346±0.6382µmol/L) than in the control group (0.584±0.2377µmol/L) and it was statistically significant ($p<0.0001$).

Conclusion: ADMA represents a new and well-characterized marker that has been associated with many traditional and novel risk factors in the setting of cardiovascular risk. So, an elevation of ADMA levels of patients with hyperthyroidism compared with healthy controls may contribute to some cardiovascular alterations.

Keywords: ADMA= Asymmetric dimethylarginine, eNOS = endothelial nitric oxide synthase, NO= nitric oxide, ELISA= enzyme-linked immunosorbent assay, *p* = Probability of observing a test statistic.

Introduction

Cardiovascular manifestations are frequent findings in patients with thyroid hormone disorders, especially those with hyperthyroidism.[(1)] There is abundant evidence that the endothelium plays a crucial role in the maintenance of vascular tone and structure. Endothelium-derived mediators help in vascular homeostasis. One of the major endothelium-derived vasoactive mediators is nitric oxide (NO), an endogenous messenger molecule formed in healthy vascular endothelium from the amino acid precursor l-arginine. It is released in response to shear stress and plays an important role in flow-mediated dilatation.[(2)] Asymmetric l-arginine (ADMA), a guanidino-substituted analogue of l-arginine, is synthesized endogenously and can act as inhibitor of NO synthase, the enzyme responsible for the formation of NO from l-arginine.[(3)] One mechanism that explains the occurrence of endothelial dysfunction is the presence of elevated blood levels of asymmetric dimethylarginine (ADMA), an l-arginine analogue that inhibits NO formation and thereby can impair vascular function.[(4)] The purpose of the present study was to evaluate and compare ADMA levels in patients with hyperthyroidism free of cardiovascular risk associates such as diabetes or chronic renal failure with further comparison with healthy control subjects.

Methodology

Present study took place in the Department of Biochemistry, Victoria Hospital, attached to Bangalore Medical College and Research Institute, Bangalore with sixty subjects (n=60), thirty cases (n_1=30) and thirty age and sex matched controls (n_2=30). Thirty patients of hyperthyroidism with no past history or current symptoms of coronary or peripheral arterial disease were included in the study. The diagnoses were based on basal plasma TSH values less than 0.5 mIU/litre and were ascertained by determination of free T4 (fT4). The patients with renal failure, diabetes and severe hypertension were excluded.

Collected blood sample was allowed to clot for 30 minutes in a clean dry test tube and was subjected to centrifugation to separate the serum. The serum samples were stored in deep freezer at minus 80 degree Celsius till they were analyzed. ADMA was estimated using double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) method, whereas, TSH and fT4 were measured by immunoassay, Beckmann Coulter Access 2.

Descriptive and inferential statistical analysis had been carried out in the present study. Results are presented on mean ± SD and Student t-test (two tailed, independent) had been used to find the significance of

study parameters (p value) between two groups (inter group analysis) on metric parameters.

To ascertain Correlation between ADMA and TSH and fT4, Pearson's correlation co-efficient (r) was used.

Results

Among 30 cases, 5, 16 and 9, and among 30 controls, 7, 13 and 10 were in the age group of < 20years, 20-40 years and >40 years respectively. **(Table 1)**

Age in years	Cases		Controls		p value
	No	%	No	%	0.536
<20	5	16.66	7	23.33	
20-40	16	53.33	13	43.33	
>40	9	30.0	10	33.33	
Total	30	100.0	30	100.0	
Mean ± SD	34.13±14.53		31.73±15.07		

Table 1: Age distribution among cases and controls. The mean age of the cases is 34.13 years with an SD of 14.53 and in controls is 31.73 with an SD of 15.07 and the age difference between cases and controls are statistically not significant (>0.05).

Sex	Cases		Controls	
	No	%	No	%
Male	8	23.33	10	30.0
Female	23	76.66	21	70.0
Total	30	100.0	30	100.0

Table 2: Sex distribution among cases and controls. Women were overrepresented in both groups of patients (76.66% in the hyperthyroid group and 70.0% in the control group), and therefore, the control group was gender-matched by the inclusion of more control women than men.

Mean ± SD serum ADMA levels in cases and controls are 1.1346 ± 0.6382 and 0.584 ± 0.2377 µmol/L and the difference was found to be statistically significant (p<0.0001). (Table 3; Fig. 1)

Table 3: ADMA concentration among cases and controls

Parameter	Cases	Controls	t score	p value
ADMA (µmol/L)	1.1346±0.6382	0.584±0.2377	4.4282	<0.00001

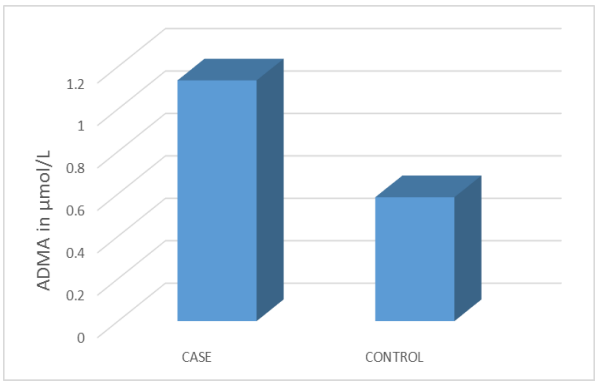

Fig. 1: Comparison of serum ADMA in the two groups studied

In the present study, serum ADMA has shown a significant positive correlation with free T4 (r = 0.5843, p=0.000698) and a negative correlation with serum TSH (r = - 0.4889, p=0.006115). (Table 4; Fig. 2, 3)

Table 4: Correlation of serum ADMA levels with individual parameters in cases

Parameters	Correlation coefficient 'r'	p value
TSH(mIU/L)	- 0.743	<0.00001
fT_4 (ng/dl)	0.966	<0.00001

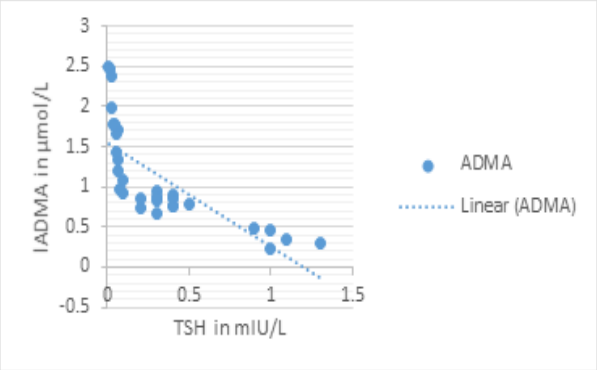

Fig. 2: Scatter plot showing negative correlation between serum ADMA and TSH which is statistically significant (p<0.00001)

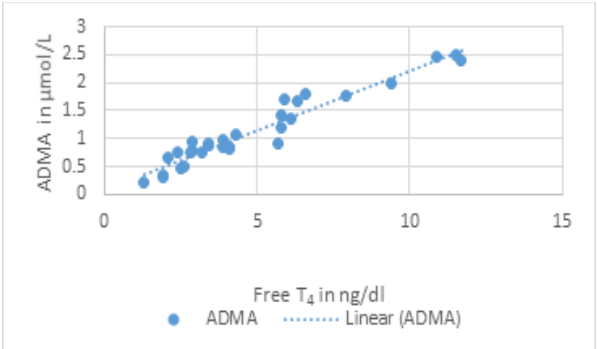

Fig. 3: Scatter plot showing positive correlation between serum ADMA and free T_4 which is statistically significant (p<0.00001)

Discussion

The present study demonstrated that significant increase in serum ADMA concentration in cases compared to controls. Serum ADMA has shown a significant positive correlation with free T_4 and a negative correlation with serum TSH in the study.

Many studies have reported a relationship between raised ADMA concentrations and cardiovascular disease. Plasma ADMA concentrations were shown high in patients with coronary artery disease (CAD),[5] peripheral arterial disease,[6] pulmonary hypertension,[7] stroke,[8] and hypertrophic cardiomyopathy.[9] ADMA levels were also significantly elevated in patients with various cardiovascular risk factors as demonstrated for hypertension,[10] hyperlipidemia,[11] and hyperhomocysteinemia.[12]

Schulze F *et al* studied 800 people with and without established coronary artery disease to determine the Coronary Artery Risk investigating the Influence of ADMA Concentration. The plasma ADMA concentration was 20% higher in the presence of established CAD and the ADMA levels increased with increasing number of cardiovascular risk factors.[13]

Valkonen *et al* did a case-control study among middle-aged men from Finland and reported a 3.9 fold increased risk of acute coronary events in subjects with highest quartile of ADMA compared to other quartiles.[5]

Lu and colleagues followed-up 153 people with stable angina undergoing percutaneous coronary intervention for a duration of 16 months during which time major cardiovascular events occurred in 51 patients. An increased risk of cardiovascular events was noted with increasing levels of ADMA.[14]

In a study of women in Gothenberg, baseline ADMA levels were measured in 880 healthy women who were followed-up for 24 years showed that a 0.15µmol /l increase in baseline ADMA levels was associated with 30% increase in cardiovascular risk and 30% increase in fatal cardiovascular disease approximately after adjustment for conventional cardiovascular risk factors.[15]

As hyperthyroidism has been associated with high frequency in clinical presentation of cardiovascular complications like tachycardia, systolic hypertension, atrial fibrilation, heart failure it shows high probablity of cardiovascular mortality and morbidity risk.[16] Patients with hyperthroidism show increased mortality by 20% and the major causes of death are cardiac problems.[17] Clinical studies revealed that dysfunction of endothelial L-arginine/ NO pathway seems to be the possible cause of such complications by which several cardiovascular risk factors mediate their deleterious effects on the vascular wall. Direct relationship between plasma T4 and ADMA levels might reflect an impaired l-arginine-NO pathway in hyperthyroidism that could be related to some of its cardiovascular alterations.[18]

ADMA is known to be a mediator molecule of the adverse vascular effects and also markers of cardiovascular risk. It is synthesized when arginine residues in the nuclear proteins are methylated through the action of the protein arginine methyltransferases (PRMTs) through a posttranslational change which adds one or two methyl groups to the nitrogens of the guanidine incorporated into the proteins.This specific enzyme protein arginine *N*-methyltransferase (protein methylase I) has been shown in many studies to methylate internal arginine residues in a variety of polypeptides. Catabolism of these polypeptides generates ADMA.[19] ADMA is metabolized by the action of the dimethylarginine dimethylaminohydrolases (DDAHs). Factors like oxidative stress contribute to diminishing DDAH activity, leading to an elevation of ADMA levels. This results in ADMA concentrations and, subsequently, to decreased endothelial nitric oxide–mediated nitric oxide production and endothelial dysfunction.[20]

An elevation of plasma ADMA in patients with hyperthyroidism can be because of an increased synthesis of ADMA, or a decreased enzymatic hydrolysis. Thyroid hormone up-regulates protein methylase I activity,[21] offering a putative mechanisms for elevated ADMA levels associated with hyperthyroidism. Also, it could be hypothesized that hyperthyroidism would decrease DDHA activity through increased production of oxygen free radicals and oxidative stress.[22]

Conclusion

The present study demonstrated an elevation of serum ADMA levels of patients with hyperthyroidism associated with a reduction in NO production, and its strong correlations with TSH and free T_4 show that assessment of ADMA might aid cardiovascular risk assessment. This biomarker identifies early detection of endothelial dysfunction, and thus can be used to identify individuals at high cardiovascular risk even in early stage, apart from traditional risk factor and inflammatory biomarkers.

References

1. Klein I,Ojamma K " Thyroid hormone and the cardiovascular system" N Engl J Med (2001) 344,501-509.
2. Latika Sibal, Sharad C Agarwal, Philip D Home and H Boger, "The Role of Asymmetric Dimethylarginine (ADMA) in Endothelial Dysfunction and Cardiovascular Disease" Current Cardiology Reviews (2010) 6,82-90.
3. Vallance P, Leone A, Calver A, Collier J, Moncada S, "Accumulation of an endogenenous inhibitor of nitric oxide synthesis in chronic renal failure " Lancet (1992) 339,572-575.
4. Rainer H Boger " Asymmetric dimethylarginine(ADMA) and cardiovascular disease: insights from prospective clinical trials" Vascular medicine, SAGE Publications (2005)10,19-25.

5. Valkonen VP, Paiva H, Salonen JT, *et al* "Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine" Lancet (2001)358,2127-8.
6. Mittermayer F, Krzyzanowska K, Exner M, *et al* "Asymmetric dimethylarginine predicts major adverse cardiovascular events in patients with advanced peripheral artery disease" Arterioscler Thromb Vasc Biol(2006) 26,2536-40.
7. Gorenflo M, Zheng C, Werle E, Fiehn W, Ulmer H E "Plasma levels of asymmetrical dimethyl-L-arginine in patients with congenital heart disease and pulmonary hypertension" J cardiovascular Pharmacol(2001) 37,489-92.
8. Yoo J H, Lee S C "Elevated levels of plasma homocyst(e)ine and asymmetric dimethylarginie in elderly patirnts with stroke" Atherosclerosis (2001)158,425-30.
9. Dimitrow P P, Undas A, Bober M, Tracz W, Dubiel JS "Plasma biomarkers of endothelial dysfunction in patients with hypertrophic cardiomyopathy" Pharmacol Rep (2007) 59,715-20.
10. Francesco Perticone, Angela Sciacqua, Raffaele Maio, Maria Perticone, Renke Mass, Rainer H. Boger *et al* "Asymmetric Dimethylarginine, L-Arginine, and Endothelial Dysfunction in Essential Hypertension" Journal of the American College of Cardiology (2005) 46,518-523.
11. Lundman P, Eriksson M J, Stuhlinger M, Cooke J P, Hamsten A, Tornvall P "Mild-to-moderate hypertriglyceridemia in young men is associated with endohelial dysfunction and increased plasma concentrations of asymmetric dimethylarginine" J Am Coll Cardiol (2001) 38,111-116.
12. Dayal S, Lentz S R "ADMA and hyperhomocysteinemia" Vasc Med(2005)10,S27-33.
13. Schulze F, Lenzen H, Hanefeld C *et al* "Asymmetric dimethylarginine is an independent risk factor for coronary heart disease: results from the multicenter Coronary Aetery Risk Determination investigating the influence of ADMA concentration (CARDIAC) study" Am Heart J (2006) 152,491-498.
14. Lu T M, Ding Y A, Lin S J, Lee W S, Tai H C. "Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention" Eur Heart J(2003) 24,1912-9.
15. Leong T, Zylberstein D, Graham I *et al* "Asymmetric dimethylarginine independently predicts fatal and nonfatal myocardial infarction and stroke in women: 24-year follow-up of the population study of women in Gothenberg" Arterioscler Thromb Vasc Biol (2008) 28,961-7.
16. Sibel Ertek, Arrigo F. Cicero "Hyperthyroidism and cardiovascular complications: a narrative review on the basis of pathophysiology" Arch Med Sci (2013) 9,944-952.
17. Brandt F, Green A, Hegedüs L, Brix TH"A critical review and meta-analysis of the association between overt hyperthyroidism and mortality. Eur J Endocrinol" (2011) 165, 491-7.
18. Vallance P, Leone A, Calver A, Collier J, Moncada S " Endogenous dimethylarginine as an inhibitor of nitric oxide synthesis. J Cardiovasc Pharmacol" (1992)20,S60–S62.
19. Wada K, Inoue K, Hagiwara M " Identification of methylated proteins by protein arginine N-methyltransferase 1, PRMT 1,with a new expression cloning strategy" Biochim Biophys Acta (2002) 1591,1-10.
20. Cooke J P "Does ADMA cause endothelial dysfunction?" Arterioscler Thromb Vasc Biol (200) 20,2032-2037.
21. Fujimoto K1, Matsuu K, Hu-Wang E, Lu R, Shi Y B "Thyroid hormone activates protein arginine methyltransferase 1 expression by directly including c-Myc trnscription during Xenopus intestinal stem development" J Biol Chem (2012) 287,10039-50.
22. Antonio Mancini, Chantal Di Segni, Sebastiano Raimondo,Giulio Olivieri, Andrea Silvestrini, Elisabetta Meucci and Diego Curro "Thyroid Hormones, oxidative stress and inflammation" Mediators Inflamm (2016) 1,1-12.