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A COMPARISON OF ASYMMETRIC DIMETHYL ARGININE AND NITRIC OXIDE IN NORMOTENSIVE AND HYPERTENSIVE PATIENT IN TERTIARY CARE HOSPITAL

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Abstract

Background: Hypertension affects over one billion individuals and doubles the risk of cardiovascular diseases. It is linked to dietary sodium chloride, low calcium and potassium intake, alcohol, psychosocial stress, and decreased physical activity. Uncontrolled hypertension increases the risk of heart, brain, and kidney problems. An estimated 1.13 billion people worldwide have hypertension, with global targets to reduce prevalence by 25%. The study aims to assess serum ADMA and NITRIC OXIDE levels in normotensive and hypertensive patients and analyze the correlation between these levels. **Material and Methods:** The observational study was conducted with 100 cases of 18 to 65 years of age diagnosed hypertension (SBP>135mmHg, DBP> 85mmHg) and 100 controls were apparently healthy subjects was included for this study. Under sterile condition, 5ml of peripheral venous blood as a postprandial sample was collected to test ADMA using the competitive-ELISA principle and The Nitrogen Oxide Assay Kit using colorimetric method. **Results:** Majority were in the age group of 31 – 60 years which is 33% and 53.5% were females. The study found a significant difference in ADMA and NITRIC_OXIDE values between normal and hypertensive patients there was also a significant negative correlation between ADMA and Nitric Oxide values, with 74.7% and 60.9% significant negative correlations observed in hypertensive patients and 49.4% in normal individuals. This suggests that as ADMA increases, Nitric oxide values decrease or vice versa, and vice versa for normal individuals. **Conclusion:** High levels of ADMA are linked to acute vascular events and vascular damage, as it impairs nitric oxide activity. This impairment of endothelial function is a primary patho mechanism. The study highlights the interaction between increased plasma concentrations of ADMA and oxidative stress, leading to endothelial dysfunction. Further research is needed to evaluate potential therapeutic options to lower ADMA concentration.

Key words: *Hypertension, Asymmetric Dimethyl Arginine & Nitric Oxide*

Introduction

Elevated blood pressure affects more than one billion individuals and causes Hypertension. It doubles the risk of cardiovascular diseases. In the developed societies Blood pressure increases gradually in the first couple of decades. Blood pressure is very much related

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age. It tracks overtime in children and between adolescence and young adulthood. The likelihood of hypertension increases with age, and among individuals aged≥ 60 years, the prevalence is 65.4%.[(1)]The prevalence of Hypertension is because of the intake of dietary sodium chloride and the high intake of sodium chloride paves way for the age related increase in blood pressure. Low dietary consumption of calcium and potassium may lead to the risk of Hypertension. The urine sodium to potassium ratio (an index of both sodium and potassium intakes) is a stronger correlate of blood pressure than either sodium or potassium. Consumption of alcohol, psychosocial stress, and the decreased level of physical activity may also increase the level of hypertension. [(2)] The AHA issued guidelines in November2017 that define hypertension as blood pressure that is consistently higher than130 / 80 mmHg. The prevalence of hypertension steadily increased with age, increasing from 24.9% among 30 to 49 years of age group to 61.7 %, among those more than 60years. The national estimate of hypertension awareness in India is 44.7%. ADMA (Asymmetric Dimethyl Arginine) occurs naturally in endogenous inhibitor of NO (Nitric oxide) synthase. ADMA reduces NO production and this could lead to endothelial dysfunctions and cardiovascular events. Studies have revealed that the increased concentrations of ADMA present in some patho-physiological conditions are linked with other elements, leading to the increased risk of atherosclerosis, such as raising age, hypercholesterolemia, hypertension and hyperglycaemia. [(3)]

Uncontrolled Hypertension or elevated blood pressure is a serious medical condition that significantly increases the risk of heart, brain and kidney. An estimated 1.13 billion people worldwide have hypertension. Most 2/3 are of people living in low and middle income countries. In 2015, 1 in 4 men and 1in 5 women had hypertension. One of the global targets for non-communicable diseases is to reduce the prevalence of hypertension by 25%. In 2016,[(4)] WHO and the United States centre for disease control (CDC) and prevention launched the global heart initiative to support governments to prevent and treat cardiovascular diseases. The potential causal relationship between elevated ADMA and cardiovascular events and mortality in humans can only be revealed in prospective clinical studies.[(4)]

Aims and Objective:

- The study aims to determine serum levels of ADMA and NITRIC OXIDE in normotensive and hypertensive patients,
- To analyse the correlation between ADMA and NITRIC OXIDE levels normotensive and hypertensive patients.

Materials And Methods

The observational study was conducted during the period from January 2019 to December 2019. Study population had known and unknown hypertension subjects as cases and controls were apparently healthy subjects.

STUDY POPULATION

Cases group:

- This group consisted of 100 patients with hypertensive patients were attending OP in the hypertension and diabetic clinic Government Chengalpattu medical college &Hospital Chengalpattu.

INCLUSION CRETERIA

- 100 subjects of 18 to 65 yrs. of age diagnosed hypertension (SBP>135mmHg, DBP> 85mmHg) was included for this study.
- 100- age, sex and risk factor matched healthy controls with systolic BP<135mmHg, DBP<85 mmHg.

EXCLUSION CRITERIA:

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- Those who are not willing to participate in the study.
- Subjects with co-morbid conditions like diabetes mellitus,
- Cirrhosis and subjects on nitric oxide derivatives like GTN, isosorbide dinitrate, isosorbide mononitrate, etc., will be excluded.

ANALYSIS PLAN:

- The statistical analysis will be performed by T-test, ANOVA, linear regression, Bland Altiman plot etc.
- The study was approved by the Institutional Ethical Committee of CHENGALPATTU MEDICAL COLLEGE, Chengalpattu.
- After a full explanation of the study a written informed consent was obtained from each participant.

SAMPLE COLLECTION:

- UNDER STERILE condition, 5ml of peripheral venous blood as a postprandial sample was withdrawn using sterile disposable syringes from all the study subjects.

SERUM SEPARATION:

- The sampled tube was centrifuged at 2500 rpm for 20 minutes. Then serum was separated with sterile pipet carefully and transferred to 2ml in eppendorf, and stored at -2to -8*C freeze.

TEST ASSAY PROCEDURE FOR ADMA

The ADMA testing procedure uses the competitive-ELISA principle, where a microtiter plate is pre-coated with ADMA. The ADMA in the sample or standard competes with a fixed amount of ADMA on the solid phase supporter for sites on the Biotinylated detection Ab specific to ADMA. Excess of conjugate and unbound samples are cleansed from the plate, and Avidin conjugated to Horse Radish Peroxidase (HRP) is added to each well and incubated. A TMB substrate solution is then added to each well. The enzyme substrate reaction terminates with the inclusion of stop solution, and the change of color is measured spectrophotometrically at a wavelength of 450nm ± 2nm. The concentration of ADMA in the samples is then valued by comparing the OD of the samples to the standard curve. The preparation process involves setting all reagents to room temperature, following the microplate reader manual for set-up, and preparing wash buffer. Standard working solution is prepared by centrifuging the standard at 10000 × g for 1min, adding 1.0 ml of reference standard and sample diluent, and mixing thoroughly with the pipette. The assay procedure involves adding standard working solution to the first two columns, adding samples to other wells, adding 50 µl of Biotinylated Detection Ab working solution, and covering the plate with a sealer. The decant solution is aspirated and repeated five times. Substrate reagent is added to each well, and the optical density (OD value) of each well is determined using a micro plate reader set to 450nm.

NITRIC OXIDE ASSAY KIT

The Nitrogen Oxide Assay Kit is a colorimetric method used to measure the nitrogen oxide (NO) content in serum. Nitric oxide is a reactive free radical with a short half-life and is produced by various cells in the blood. It can be calculated indirectly by analyzing the concentration of nitrate and nitrite. The kit consists of several components, including a sulphate solution, an aqueous alkali, a chromogenic agent, and a sodium nitrite standard solution. The chromogenic agent is prepared by mixing reagents 3:3:2, and the sodium nitrite standard solution is prepared by diluting reagent 6 with distilled water. The results are then calculated using the OD value at 550 nm using a cuvette. The kit is recommended for use in blood tests to detect nitric oxide levels.

ESTIMATION OF TOTAL CHOLESTEROL IN SERUM (CHOD-POD METHOD)

The study uses a cholesterol esterase-cholesterol oxidase CHOD-POD method to measure cholesterol concentration in a sample. The intensity of the red complex is directly

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proportional to the sample's cholesterol concentration. The reagent composition includes buffer, cholesterol oxidase, esterase, peroxidise, aminoantipyrine, phenol, and cholesterol standard.

Results

An observational study was conducted to among 100 hypertensive patients along with 100 normal participants to assess their ADMA and nitric oxide values between the groups. Figure 1 describes the age distribution of study participants and majority were in the age group of 31 – 60 years which is 33%.

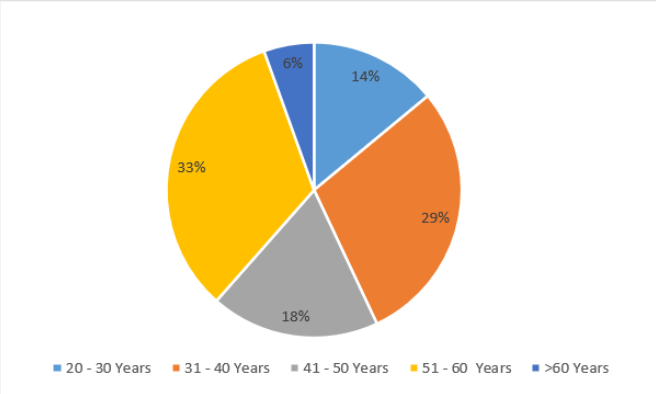

Figure 1: Age distribution of study participants

Table 1: Comparison of age and gender distribution between Normal and hypertensive patients

	Normal	**Hypertensive**	**Total**	**Chi square test value & P value**
Age (Years)				
20 - 30 Years	26	2	28	Chi sq = 45.09 P <0.001
31 - 40 Years	38	20	58	
41 - 50 Years	15	22	37	
51 - 60 Years	16	50	66	
>60 Years	5	6	11	
Gender				
Male	48	45	93	Chi sq = 0.18 P = 0.67
Female	52	55	107	
Total	**100**	**100**	**200**	

Table 1 explains that there was significant difference in age group between normal and hypertensive patients. In the age of 20 years to 40 years hypertensive patients were lesser; but as age increased majority were hypertensive patients from age 41 years and above which was statistically significant with p<0.001. **Among 100 participants in** normal group, 48% were male and 52% were female where in hypertensive 45% were male and 55% female participants hence there was no significant difference in gender between normal and hypertensive patients (p>0.05).

Table 2: Comparison of ADMA and Nitric oxide values for Normal and Hypertensive patients

	Group	**N**	**Mean**	**Standard Deviation**	**t value**	**P**

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ADMA	Normal	100	301.69	154.54	16.63	**<0.001**
	Hypertensive	100	661.13	151.12		
NITRIC_OXIDE	Normal	100	27.45	5.43	10.73	**<0.001**
	Hypertensive	100	17.76	7.23		

Table 2 describes, in normal group the average value of ADMA 301.69 (SD 154.54) and in hypertensive group average was 661.13 (SD 151.12) which implies that there was significant difference in ADMA values between normal and hypertensive patients with t value of 16.63 ($p<0.001$). In normal group the average value of Nitric oxide was 27.45 (SD 5.43) and in hypertensive group average was 17.76 (SD 7.23) which implies that there was significant difference in ADMA values between normal and hypertensive patients with t value of 10.73 ($p<0.001$).

Table 3: Comparison of ADMA and NITRIC OXIDE values between Normal, HT and DM with HT patients

	N	**Mean**	**Standard Deviation**	**One way ANOVA & p value**
ADMA				
Normal	100	301.69	154.54	F = 139.25 **p<0.001**
Hypertensive	75	671.58	147.99	
DM & HT	25	629.79	159.09	
Total	200	481.41	236.016	
NITRIC OXIDE				
Normal	100	27.45	5.43	F = 57.58 **p<0.001**
Hypertensive	75	17.51	7.14	
DM & HT	25	18.50	7.60	
Total	200	22.60	8.02	

Table 3 describes, in normal group the average value of ADMA was 301.69 (SD 154.54) and in only hypertensive group average was 671.58 (SD 147.99) and Hypertensive along with Diabetes Mellitus group was 629.79 (SD 159.09). To compare more than two group difference one way ANOVA was used at 5% level of significance and the results showed that there was significant difference in ADMA values between normal and study groups with F value of 139.25 ($p<0.001$). The mean comparison were depicted in Graph 6.

Table 3 describes, in normal group the average value of Nitric oxide was 27.45 (SD 5.43) and in hypertensive group average was 17.51 (SD 7.14) and Hypertensive along with Diabetes Mellitus group was 18.5 (SD 7.6). To compare more than two group difference one way ANOVA was used at 5% level of significance and the results showed that there was significant difference in Nitric oxide values between normal and study groups with F value of 57.58 ($p<0.001$). The mean comparison were depicted in Graph 7.

Table 4: Overall Pearson Correlation between ADMA and Nitric oxide values among both study participants

Correlations		**NITRIC_OXIDE**
ADMA	Pearson Correlation	-0.747
	Sig. (2-tailed)	**0.0001**
	N	200

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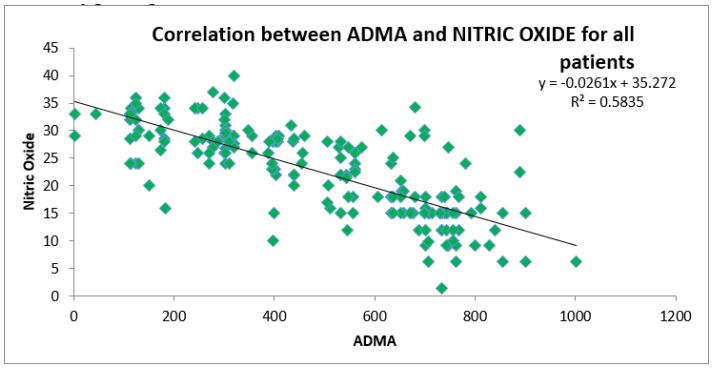

Figure 2: Overall Pearson Correlation between ADMA and Nitric oxide values among both study participants

Figure 2, the scatter plot describes that there was 74.7% significant negative correlation between ADMA and Nitric Oxide values (p =0.0001) which implies that as ADMA increases Nitric oxide values decreases or vice versa. The strength of relationship was established by simple linear regression equation Y = 35.272 - 0.0261X with R^2 = 0.5835.

Table 9: Correlation between ADMA and Nitric oxide among Hypertensive and normal patients

Correlations		**NITRIC_OXIDE**	
		Hypertensive patients	**Normal individuals**
ADMA	Pearson Correlation	-0.609	-0.494
	Sig. (2-tailed)	**<0.001**	**<0.001**
	N	100	100

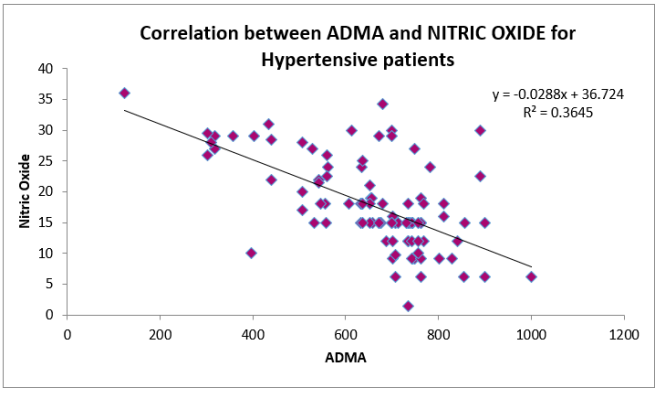

Figure 3: Correlation between ADMA and Nitric oxide among Hypertensive patients

Figure 3, the scatter plot describes that there was 60.9% significant negative correlation between ADMA and Nitric Oxide among hypertensive patients (p =0.0001) which implies that as ADMA increases Nitric oxide values decreases or vice versa. The strength of relationship was established by simple linear regression equation Y = 36.724 - 0.0288X with R^2 = 0.3645.

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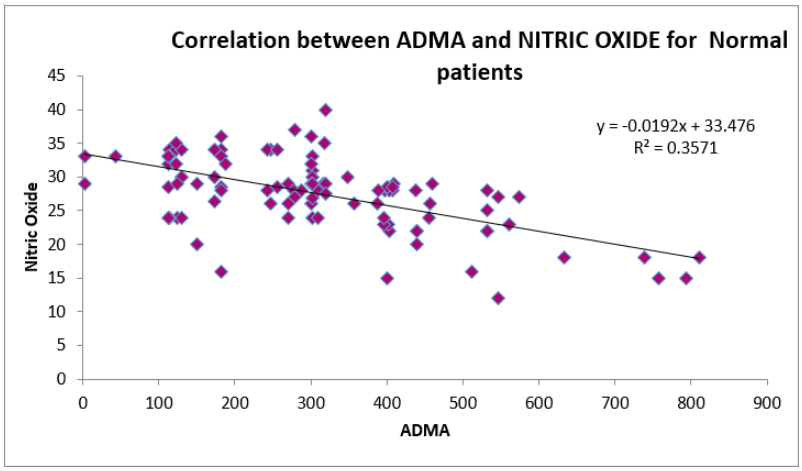

Figure 4: Correlation between ADMA and Nitric oxide among Normal individuals

Graph 4, the scatter plot describes that there was 49.4% significant negative correlation between ADMA and Nitric Oxide among normal individuals (ie) control group (p =0.0001) which implies that as ADMA increases Nitric oxide values decreases or vice versa. The strength of relationship was established by simple linear regression equation Y = 33.476 - 0.0192X with R^2 = 0.3571.

Discussion

The newly diagnosed and untreated hypertension had increased plasma ADMA levels and depressed systemic NO formation compared to normotensive controls.

This study demonstrated a well-known association between BP and vascular resistance. Because ADMA is an endogenous competitive receptor of NO synthase and can modulate endogenous vasodilator NO production. There were theoretical reasons to assume that plasma ADMA and nitrate (metabolite of NO) are also associated with BP and other hemodynamic regulation. However, our extensive investigation package indicated no apparent association between plasma ADMA or nitrate and hemodynamic regulation in our study group.

In a clinical setting, only significantly elevated plasma ADMA levels may modulate NO production and are independent predictors of vascular functions. Such high plasma ADMA values may be related, for example, to dysfunctional ADMA-degrading enzymes, DDAH-1 and DDAH-2 because of specific genetic mutations or renal dysfunction antihypertensive treatment has reduced ADMA levels in these subjects as it has been demonstrated at least for angiotensin-converting enzyme (ACE) inhibitors and angiotensin II AT 1 receptor blockers in earlier studies.

Therefore, suggesting that ADMA may have a dual role in the regulation of BP. A suggested earlier, high ADMA levels, for example, because of genetic reasons, may cause elevated BP levels in some persons, whereas in others ADMA levels could be lowered, for example, because of hypertension caused by down regulation of the DDAH enzymes.

ADMA is synthesized from L-arginine by the action of protein methyl transferase with S-adenosyl methionine as the methyl donor, as a NOS inhibitor. ADMA is an important regulator of vascular function. Whilst ADMA is widely present, liver and kidney are the major sites of ADMA production.[3]

The metabolic regulation of L-arginine and ADMA provides a stable ratio between these two variables such that each competes for DDAH and this then ensures NO homeostasis. The functional role of L-arginine on ADMA may elaborate on the unexpected outcomes in the studies of L-arginine supplementation.

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Many studies have documented increased ADMA levels in renal and hepatic failure, and in vascular disease and also that levels are increased in association with increased circulating homocysteine levels.(5) ADMA has been referred to as a new risk factor. However, even mild reductions in renal function can result in increases of both ADMA and homocysteine.

An additional finding for ADMA is that it is increased in smokers, independent of renal function and therefore contributes to the risk of vascular disease in those who smoke. ADMA might be a marker of vascular damage.(6)

Conclusion

Based on the above study, high levels of ADMA seem to associate with the risk of acute vascular events and ADMA might be a marker of vascular damage. Given the fact that ADMA impairs nitric oxide activity, it has been widely assumed that ADMA- induced impairment of endothelial function is a prime underlying patho mechanism.

The interaction between increased plasma concentrations of the endogenous eNOS inhibitor ADMA and increased oxidative stress within the vasculature and subsequently development of endothelial dysfunction is seen. The further importance is of elevated ADMA plasma concentrations causing endothelial dysfunction and increased oxidative stress and to evaluate possible therapeutic options to lower ADMA concentration.

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