Asymmetric dimethylarginine as a prognostic marker for cardiovascular complications in hypertensive patients

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Abstract Hypertension has a serious harmful effect on the physiological and biochemical functions of heart that end with the appearance of cardiovascular diseases. Asymmetric dimethylarginine (ADMA) has evolved as an important regulator of nitric oxide (NO) synthesis. The relationship between ADMA and essential hypertension has been scarcely explored. This study is aiming to investigate the physiological, pathological, and biochemical roles of plasma ADMA in relation to serum nitric oxide and also the relation between ADMA and NO with other traditional cardiovascular risk factors in hypertensive patients.

Methods: The study was designed as a prospective case-control study which included 60 hypertensive cardiovascular patients and 10 healthy volunteers as a control group. The patients were divided into four groups: hypertension with cardiovascular complications, uncontrolled hypertension (blood pressure >139/89), controlled hypertension, and control group. Levels of ADMA and nitric oxide were assessed and statistically correlated to other clinical and laboratory values [cholesterol, triglycerides, urea, creatinine, and fasting and postprandial blood sugar].

Results: The plasma ADMA level in group 1 was significantly increased (2.29 ± 0.06 μmol/L) compared to control group (0.55 ± 0.05 μmol/L) P = 0.001 and also serum NO level was significantly decreased in this group (11.65 ± 0.75 μmol/L) compared to control group (52.11 ± 1.43 μmol/L) P = 0.001. This group had variable complications, e.g., ischemic heart disease, pulmonary hyperten-
1. Introduction

Hypertension has a serious harmful effect on the physiological and biochemical functions of heart that ended with the appearance of cardio-vascular diseases. Endothelium-mediated regulation of vascular tone and vascular structure has been a fascinating topic in cardiovascular medicine since the first discovery in 1980 by Furchgott, who stated that the vascular relaxation in response to acetylcholine critically depends upon the presence of an intact vascular endothelium. Nitric oxide (NO) was discriminated to be an elusive mediator (previously named endothelium-derived relaxing factor and released by healthy endothelium) that causes vascular dilatation, which synthesized within the endothelium from amino acid l-arginine by enzyme nitric oxide synthetase.3

Endothelial dysfunction occurs whenever the biological functions of NO are impaired, be it at the level of NO generation, during its diffusion from endothelial cells to its target cells in the subendothelial layers of the vascular wall or to the cells of the flowing blood, or within the subsequent signaling cascade that links NO to its cellular functions, have all been shown to be involved in the phenomenon called endothelial dysfunction under varying conditions.4

Asymmetric dimethylarginine (ADMA) has evolved as an important regulator of NO synthesis in recent years. ADMA is an endogenous inhibitor of NO synthetase. Several cell types including human endothelial and tubular cells are capable of synthesizing and metabolizing ADMA. Also it is formed during proteolysis of methylated proteins and removed by renal excretion or metabolic degradation by the enzyme dimethylarginine dimethylaminohydrolase (DDAH) to l-citrulline and dimethylamine. Elevated levels of ADMA have been reported in many conditions to be associated with a high cardiovascular risk.5

The relationship between ADMA and essential hypertension has been scarcely explored. Plasma ADMA levels was measured and coherently found to be higher in hypertensive patients, than in normotensive healthy subjects, particularly in salt-sensitive individuals.5 However, in none of these studies the relationship between ADMA and endothelial function was tested. In one study, urinary nitrate excretion was reduced concomitantly with elevated ADMA plasma levels in patients with essential hypertension, suggesting that systemic NO production was impaired in these patients.1

This study is aiming to investigate the physiological, patho-
logical, and biochemical roles of plasma ADMA in relation to serum nitric oxide and also the relation between ADMA and NO with other traditional cardiovascular risk factors in hypertensive cardiovascular patients.

2. Patients and methods

After local ethics committee approval and a written consent the study was conducted as a prospective case-control study which included 60 consecutive hypertensive cardiovascular patients, who were admitted to Critical Care Department of Cairo University and 10 volunteers as control. Our study population has been categorized into four groups: group I consisted of twenty hypertensive patients with cardiovascular complications. Group II had 20 patients with uncontrolled hypertension with or without treatment and there were no cardiovascular complications. Group III, which had 20 patients with controlled hypertension defined as blood pressure below 139/89, who had a regular anti hypertensive drugs. Fourth group consisted of 10 volunteers as control.

The following data were obtained: history analysis and clinical examination, then blood samples were collected from all patients. A part of blood samples collected on heparin to evaluate ADMA, the remaining samples were collected without anticoagulant and separated sera were stored at 4°C pending various biochemical assays.

1. Determination of serum nitric oxide level: was estimated in serum according to Griess reaction using a commercial kit purchased from Cayman Chemical Company, Ann Arbor, USA.
2. Determination of plasma asymmetric dimethylarginine (ADMA): was determined using commercial kits purchased from American Laboratory Products Company (Alpco Diagnostics), USA and according to the method of Schulze et al.

Sunrise absorbance reader (TECAN) was used to determine plasma ADMA and serum nitric oxide levels. APEL PD-303 S spectrophotometer was used to measure: total cholesterol, triglycerides, urea, creatinine, and fasting and postprandial blood sugar.
2.1. Statistical analysis

The data were coded and computed on a statistical package for social sciences SPSS version 11.5 for windows for statistical analysis. Continuous variables were evaluated using t-test and categorical variables using X². Pearson correlation coefficient \( r \) test was done to examine strength and direction of correlation between two variables. Correlation was weak if \( r < 0.3 \), moderate if \( 0.3 < r < 0.6 \) and strong if \( r > 0.6 \). \( P \) value < 0.05 was considered significant.

3. Results

The population in our study had no significant difference between the groups regarding male to female ratio, smoker/non smoker, but group I showed a significantly higher age than the other groups, the demographic data are illustrated in Table 1. Patients in group I had cardiovascular complications; 12 patients had ischemic heart disease\(^1\) (acute myocardial infarction, non ST segment elevation myocardial infarction, and unstable angina), two patients with pulmonary hypertension, one patient with pulmonary embolism, and five patients with cerebrovascular disease.

Hypertensive patients had a significant higher systolic and diastolic blood pressures compared to control group IV (Table 2). Both the systolic and diastolic blood pressures had a significant positive correlation with ADMA, while with NO there was a significant negative correlation \( r = -0.9 \) \( (P = 0.012; \text{Tables 2 and 4 and Figs. 1 and 2}) \).

Cardiovascular hypertensive groups I, II and III compared to control group (group IV) showed a statistically significant increase in mean FBS and PPBs \( (P < 0.002 \text{ and } 0.001, \text{ respectively}) \). Correlation coefficient shows a strong positive correlation between FBS and ADMA \( r = 0.9 \) \( (P = 0.002) \) and strong negative correlation between FBS and NO, \( r = -0.9 \) \( (P = 0.003) \). While PPBS had positive and negative correlations with ADMA and NO, but it was not significant \( (P = 0.055 \text{ and } 0.059, \text{ respectively, Tables 3 and 4}) \).

There was a significant increase in the mean urea and creatinine value in cardiovascular hypertensive groups I and II compared to group III and control group \( P \) value < 0.001 and 0.024, respectively. Even in hypertensive patients with cardiovascular complications (group I), urea and creatinine were highly significant compared to group II (patients with

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**Table 1** Patients demography in all studied groups.

<table>
<thead>
<tr>
<th>Group</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV (Control group)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>29–75</td>
<td>24–70</td>
<td>37–70</td>
<td>35–65</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>52.2 ± 2.5</td>
<td>50.6 ± 2.4</td>
<td>47.5 ± 2.02</td>
<td>45 ± 2.2</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>13 (65%)</td>
<td>12 (60%)</td>
<td>17 (85%)</td>
<td>6 (60%)</td>
<td>0.18</td>
</tr>
<tr>
<td>Female</td>
<td>7 (35%)</td>
<td>8 (40%)</td>
<td>3 (15%)</td>
<td>4 (40%)</td>
<td></td>
</tr>
<tr>
<td>Smoking</td>
<td>17 (85%)</td>
<td>14 (70%)</td>
<td>12 (60%)</td>
<td>3 (30%)</td>
<td>0.08</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>18 (90%)</td>
<td>19 (95%)</td>
<td>20 (100%)</td>
<td>0 (0%)</td>
<td>0.1</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>4 (40%)</td>
<td>0.15</td>
</tr>
<tr>
<td>Renal Impairment</td>
<td>20 (100%)</td>
<td>20 (100%)</td>
<td>16 (80%)</td>
<td>0 (0%)</td>
<td>0.12</td>
</tr>
</tbody>
</table>

\(^*\) Means significant to other groups.

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**Table 2** Systolic and diastolic blood pressures in all studied patients groups.

<table>
<thead>
<tr>
<th>Group I (mean ± SD and range)</th>
<th>Group II (mean ± SD and range)</th>
<th>Group III (mean ± SD and range)</th>
<th>Control group (mean ± SD and range)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP 176.85 ± 2.41^A 160–195</td>
<td>153.45 ± 1.61^A 140–165</td>
<td>130.95 ± 0.99^A 120–135</td>
<td>117.51 ± 1.66 110–125</td>
<td>0.011</td>
</tr>
<tr>
<td>DBP 102.35 ± 1.01^A 95–110</td>
<td>95.01 ± 0.67^A 90–100</td>
<td>85.35 ± 0.53^A 80–90</td>
<td>78.31 ± 1.52 70–85</td>
<td>0.011</td>
</tr>
</tbody>
</table>

SBP, systolic blood pressure; DBP, diastolic blood pressure.

\(^\text{A}\) Means that group is significant to control group.
uncontrolled hypertension without cardiovascular complications). Correlation coefficient shows a strong positive correlation between urea, creatinine and ADMA ($r = 0.99$ and $0.9$ ($P = 0.001$ and $0.002$, respectively) and negative correlation between urea, creatinine and NO ($r = 0.99$ and $0.9$ ($P = 0.002$ and $0.0018$, respectively, Tables 3 and 4).

Cholesterol and triglycerides levels show significant higher level in group I (hypertensive patients with cardiovascular complications), compared to other hypertensive groups I, II, and control group. Also groups II and III show a higher significant cholesterol and triglyceride level in relation to control group. Correlation coefficient revealed a significant positive correlation with ADMA and a significant negative correlation with NO (Tables 3 and 4 and Figs. 3 and 4).

ADMA levels was significantly higher in the hypertensive patients (groups I, II, and III) in relation to control group, even hypertensive complicated cardiovascular group and patients who have uncontrolled hypertension (groups I and II) had a significant higher level of ADMA more than patients with controlled hypertension (group III; Table 3 and Fig. 5). The correlation between plasma ADMA and age in all studied patients groups revealed a strong positive correlation $r = 0.904$ ($P < 0.049$; Fig. 6). While Fig. 7 illustrated the correlation coefficient between age and NO levels in the study population, which revealed a strong negative correlation $r = -0.927$ ($P < 0.037$).

NO levels were significantly lower in the hypertensive patients (groups I, II, and III) in relation to control group, while hypertensive complicated cardiovascular group and patients who have uncontrolled hypertension (groups I and II) had a significant lower level of NO more than patients with controlled hypertension (group III; Table 3 and Fig. 5). There was a strong inverse and significant correlation between NO and ADMA levels in the whole population ($r = -0.9$ and $P$ value $0.001$) as shown in Fig. 8.

By correlation of the laboratory tests done in our study and ADMA and NO levels; there were significant positive correlations between ADMA and all variables that serve as cardiovascular risk factors (blood sugar, cholesterol, and triglyceride) and on the other hand there were significant negative correlations between these risk factors and NO as shown in Table 4.

4. Discussion

Hypertension remains a common and serious problem, contributing in a major way to the most common causes of morbidity and mortality worldwide. Moreover, the main burdens

<table>
<thead>
<tr>
<th>Table 3</th>
<th>Laboratory results for all studied patients groups and control group.</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Group I (mean ± SD and range)</td>
</tr>
<tr>
<td>FBS</td>
<td>120.9 ± 42.00B</td>
</tr>
<tr>
<td>PPBS</td>
<td>73.98–210.11</td>
</tr>
<tr>
<td>Urea</td>
<td>122.55 ± 4.17</td>
</tr>
<tr>
<td>Creatinine</td>
<td>2.13 ± 0.05</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>404.31 ± 7.98</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>282.75 ± 5.03</td>
</tr>
<tr>
<td>NO</td>
<td>11.65 ± 0.75B</td>
</tr>
<tr>
<td>ADMA</td>
<td>2.29 ± 0.06B</td>
</tr>
</tbody>
</table>

Normal range: FBS and PPBS, fasting and postprandial blood sugar (up to 110 and 126 mg/dL, respectively), urea (10–50 mg/dL), creatinine (0.6–1.2 mg/dL), cholesterol (up to 200 mg/dL), triglyceride (up to 200 mg/dL), ADMA (0.4–0.8 μmol/L); NO, nitric oxide (35–55 μmol/L).

A Means that group is significant to control group.

B Means that group is significant to group III and control group.
associated with hypertension occur not in the relatively few
with severe disease but in the masses of patients with blood
pressure (BP) that are only minimally elevated.14,15

The main findings in this study were: ADMA – endogenous
inhibitor of eNOS – is significantly high in all hypertensive
patients, while NO was significantly low in all hypertensive
patient groups in comparison to control group. ADMA had
a significant negative correlation to endothelial function as
measured by low serum NO. High plasma ADMA concentra-
tion was observed and significantly correlated positively with
traditional cardiovascular risk factors, e.g., dyslipidemia, dia-
betes mellitus, and hypertension. While low serum NO, which
induces endothelial dysfunction was negatively correlated with
these traditional cardiovascular risk factors ($P < 0.05$).

Matsuguma et al.16 reported that the plasma level of ADMA
was correlated with blood pressure levels, which is in agreement
with our study results, as ADMA was high in all hypertensive
patients in comparison to control group with a significant
positive correlation with systolic and diastolic blood pressures
$r = 0.977$ ($P = 0.011$). However, Fiser et al.17 and Schulze et
al.18 showed no correlation between ADMA and blood pres-
sure. While, Bakris et al.,19 Dayoub et al.,20 and Jacobi
et al.21 demonstrated that several factors such as age, insulin
resistance, and dyslipidemia could affect the plasma levels of
ADMA and they noted that these confounding factors and/or
cardiac medications might attenuate the positive correlation
between plasma ADMA levels and blood pressure, which could
explain the negative clinical studies. But the age of the patients
and hypercholesterolemia in our study were significantly

| Table 4 Correlations between ADMA and NO and other laboratory results, blood pressure, and age. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| ADMA NO | ADMA NO | ADMA NO | ADMA NO |
| $r$ $P$ value | $r$ $P$ value | $r$ $P$ value | $r$ $P$ value |
| FBS 0.9 0.002 | –0.9 0.003 | PPBS 0.89 0.055 | –0.85 0.059 |
| Urea 0.99 0.001 | –0.99 0.002 | Creatinine 0.9 0.002 | –0.9 0.018 |
| Cholesterol 0.9 0.018 | –0.9 0.011 | Triglyceride 0.9 0.011 | –0.9 0.011 |
| NO –0.9 0.001 | –0.9 0.001 | SBP 0.9 0.011 | –0.9 0.012 |
| DBP 0.9 0.011 | –0.9 0.012 | AGE 0.9 0.049 | –0.9 0.037 |

Figure 3 Correlation coefficient between plasma ADMA and serum Cholesterol in all studied patients groups.

Figure 4 Correlation coefficient between serum NO and serum cholesterol in all studied patients groups.

Figure 5 ADMA and NO in the four patients groups.
positively correlated with serum level of ADMA and also had a negative significant correlation with the serum level of NO.

The cause of high plasma ADMA concentration in essential hypertension is presently unknown. Increased shear stress may trigger ADMA synthesis, and high ADMA in hypertension may therefore be an epiphenomenon of high BP. Alternatively, high ADMA may result from reduced catabolic rate secondary to DDAH inhibition brought about by oxidative stress, a well-known feature of human hypertension.

In patients with coronary atherosclerosis, endothelial vasodilatory dysfunction is not confined only to the epicardial conductance vessels but also extends to the coronary resistance vessels and microcirculation. Excessive inactivation of NO due to increased oxidative stress occurs in smokers, diabetics and in hypercholesterolemia. At any level of total and LDL cholesterol, the small dense LDL particle is the main culprit, which is easily prone to lipid peroxidation and thereby adheres to the endothelium. This can explain what we found in our study that hypercholesterolemia was significantly higher in group I (hypertensive patients with cardiovascular complications) more than the other groups. Also FBS and PPBS were significantly high in groups I and II (patients with complicated hypertension and uncontrolled BP) in comparison to group III (controlled BP) and control group. So high significant levels of serum cholesterol, FBS, and PPBS could be a reason for the significant reduction of the level of serum NO in the groups I and II.

In our study; ADMA showed a significant correlation with blood glucose level and there were similar findings from animal models and clinical studies that lead to the suggestion that hyperglycemia may increase plasma ADMA levels concomitantly with reduced ADMA metabolism by DDAH. However, they leave open the question of whether glucose itself or rather insulin is the agent that affects DDAH activity. In cultured endothelial cells hyperglycemia-induced oxidative stress was associated with reduced DDAH activity, resulting in elevated ADMA concentrations in conditioned media and reduced NO formation.

Diminished NO bioactivity may cause constriction of coronary arteries during exercise or during mental stress and contribute to provocation of myocardial ischemia in patients with coronary artery disease. Additionally, diminished nitric oxide bioactivity may facilitate vascular inflammation that could lead to oxidation of lipoproteins and foam cell formation, the precursor of the atherosclerotic plaque. Accordingly, there is a causal relationship between improved endothelial function and reduction in myocardial ischemia and acute coronary events and this was proved in our study, as the lowest significant serum level of NO was present in group I (patients with complicated hypertension) to the control group.

Heibashy and Abdel Moniem reported that; the endothelial dysfunction in hypercholesterolemic individuals could be...
related to plasma concentration of ADMA, which is a strong independent predictor of overall mortality and cardiovascular outcome in hemodialysis patients, and increased plasma concentrations of ADMA have been reported in hypertension and pre-eclampsia. It is increased early enough during atherogenesis to be eligible as a causative factor.\(^{12}\) Even small changes in extracellular fluid have been shown to correspond to large enough changes in intracellular ADMA concentration that can affect NOS activity.\(^{18}\) A study in healthy volunteers demonstrated that intravenous ADMA infusion at a dose resulting in pathophysiological concentrations augments peripheral and renovascular resistance and arterial pressure.\(^{29}\) This led to suggestion that; displacing ADMA with excess L-arginine\(^{30}\) could be useful in hypertension, pre-eclampsia, and coronary heart disease. Boger et al.\(^{31}\) and Boger and Ron\(^{32}\) stated that in offspring of patients with essential hypertension in whom endothelial dysfunction is present, could be reverted to normal by intra-brachial L-arginine. These results suggest that impairment in eNOS production precedes the onset of hypertension that could be due to increase in the levels of ADMA. Also high plasma ADMA concentration was observed in the presence of hyperhomocysteinemia,\(^{33}\) inducing endothelial dysfunction in some of these conditions.\(^{34}\)

Patients in groups I and II show significant high urea and creatinine in comparison to controlled hypertensive patients and control volunteer group. Urea and creatinine were positively correlated with serum ADMA level and this can be explained by Asagami et al.,\(^{35}\) Achan et al.,\(^{36}\) and Kielstein et al.\(^{37}\) where they stated that; elevated ADMA in the context of renal insufficiency occurs in a variety of ways, probably because the DDAH activity – which causes metabolic degradation to ADMA – is affected. Zoccali et al.\(^{38}\) demonstrated an analysis of a large group of patients with end-stage renal failure that showed an inverse relationship between ADMA levels and left ventricular (LV) ejection in a multivariate analysis. ADMA was shown to be an independent and strong predictor of LV ejection fraction. These findings raise the possibility that a causal relationship between ADMA and LV dysfunction may exist.

Cooke\(^{39}\) reported that; traditional cardiovascular risk factors impair endothelial vasodilator function by causing the accumulation of ADMA. Furthermore, by blocking NO generation, ADMA initiates and promotes processes involved in atherogenesis, plaque progression, and plaque rupture. So, ADMA should be as a marker, which is a biochemical factor mediating the adverse vascular effects of many other risk factors and markers. Also Makino et al.\(^{40}\) stated that; ADMA may emerge as a prognostic marker positively associated with risk like in other atherosclerotic vascular diseases. This could be caused by changes in the relative contribution of other traditional cardiovascular risk factors, ADMA, and oxidative stress to the progression of vascular diseases.

In conclusion, ADMA is elevated in hypertensive patients. Elevated ADMA concentrations are associated with impaired endothelium functions, which are demonstrated by NO reduction in the sera of hypertensive patients. ADMA is correlated positively with the traditional cardiovascular risk factors. Also there was a strong significant negative correlation between NO and ADMA levels in the whole hypertensive groups.

References