Asymmetric Dimethyl Arginine and nitric oxide in chronic renal disease patients with cardiac and non cardiac complications

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Abstract: Asymmetrical dimethylarginine (ADMA) is inhibitor of nitric-oxide synthase, which has been linked to endothelial dysfunction and atherosclerosis in the general population, is raised in patients with end-stage renal disease and could contribute to the high cardiovascular risk in patients with chronic renal failure. Aim of the work, the aim of this study is to evaluate the levels of ADMA and NO as early predictor of cardiac complication in chronic renal disease patients. Patient and methods. Thirty patients with chronic renal failure under hemodialysis three setions//week and fifteen apparently healthy individuals as a control group .They were selected from internal medicine department (nephrology unit), Al Zahra university hospital underwent. Complete history taking, full clinical examination, laboratory investigations as complete blood picture, blood urea, serum creatinine, serum Na, serum K, cholesterol, triglyceride,ECG, echocardiography, ADMA,and NO. Conclusion, Elevated plasma ADMA level in the chronic renal failure patients. Also plasma ADMA levels predict cardiovascular events in patients with chronic renal failure.

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Key words: ADMA, NO,CKD,Cardiovascular complications.

1. Introduction

Asymmetric dimethylarginines (ADMA) and symmetric dimethylarginine (SDMA) were first isolated from human urine by Kakimoto and Akazawa in 1970 . Methylarginines derive from the posttranslational methylation of *L*-arginine residues within proteins catalyzed by a family of enzymes called protein arginine methyltransferases (PRMT). (Vallance & Leiper 2004).

ADMA is thought to be a major type of endogenously generated methylated arginine that possesses the inhibitory activity of Nitric Oxide synthase (NOS) (Ueda *et al.,* 2007). ADMA inhibits all 3 isoforms of NOS, which is reversed by excess L-arginine supplementation, and its inhibitory activity on the 3 isoforms of NOS is approximately equipotent with that of monomethyl *L*-arginine (*L*-NMMA) (Boger 2007).

Nitric oxide (NO) is a very active, but short living, that is released in the circulation from endothelial cells. It is a potent vasodilator that regulates vascular .resistance and tissue blood flow. In addition, NO inhibits key processes of atherosclerosis, such as monocyte endothelial adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. Hence, endothelial dysfunction due to reduced NO availability is an early step in the course of atherosclerotic vascular disease (fliser, etal., 2003).

Nitric oxide is synthesized by oxidation of the terminal guanidine nitrogen of L-arginine by the action of the NOS. The synthesis of NO can be blocked by inhibition of the NOS active site with guanidino-substituted analogues of L-arginine, such as ADMA (Ueda *et al.,* 2007).

There has been evidence that even minor renal dysfunction is associated with high risks of cardiovascular events (Go *et al.,* 2004 : Amann *et al.,* 2006). Chronic kidney disease (CKD) is generally thought to be one of the major risk factors for cardiovascular disease (CVD). Since endothelial dysfunction is an initial step of atherosclerosis in patients with risk factors such as hypertension, diabetes and CKD reduced production and/or bioavailability of NO may link these risk factors to the events of CVD. (Zoccali, 2007),

ADMA is a potent endogenous NOS inhibitor, and its accumulation may play important roles in endothelial dysfunction, there by contributing to the development and progression of CVD and renal injury in patients with CKD (Abedini *et al.,* 2010).

Increased plasma levels of ADMA are associated with endothelial dysfunction and predict the progression to dialysis and death in patients with chronic kidney disease. ADMA has also been shown in patients with CKD to be an important risk factor for progression to end-stage renal disease and all-cause mortality. (Ravani *et al.,* 2005).

Asymmetric dimethylarginine is an endogenous competitive inhibitor of nitric oxide synthase and reduces NO generation, thus inhibiting the beneficial effect of NO on vasodilatation, arterial stiffness, and endothelial function, ADMA levels are inversely related to glomerular filtration rate (GFR) in patients with mild-to-moderate CKD, and elevated levels have been associated with CV events and death in patients receiving hemodialysis. Thus, ADMA may be a predictor for renal graft loss, CV events and all-cause mortality in patients with different stages of CKD. (fliser,etal., 2005).

Increased plasma ADMA in patients with nephropathy was believed to result from diminished renal clearance. However, new evidence suggests that more complex mechanisms are responsible for elevations in plasma ADMA. Potentially, enhanced release of ADMA from protein stores via proteolytic pathways and/or diminished activity of dimethylarginine dimethylaminohydrolase (DDAH), the only known enzyme to metabolize ADMA could lead to increased concentrations.(Bilecke *et al.*, 2009).

Aim of the work:

The aim of this study is to evaluate the levels of ADMA and NO as early predictor of cardiac complication in chronic renal disease patients.

2. Patients and Methods

Our study included 30 patients with end stage renal disease, on regular hemodialysis, and 15 healthy controls. All patients were selected from Hemodialysis unit in Al Zahraa university hospital.

Blood samples were withdrawn from all patients and controls during routine investigation of hospitalized patients. Blood sample was divided into 2 tubes, one containing EDTA for complete blood picture and the other plain tube put in water path at 37c° for 30 minute, was centrifuged for 10 minute and serum was separated and divided in to 2 tubes one tube for liver and kidney functions and lipid profile, the other stored at-20c° for ADMA and nitric oxide later on.

Patients were divided into 3 groups:

Group I: hemodialysis with cardiac complication (ischemic heart disease) group: Fifteen patients their ages averaged 51.13±8.41 (rang 37-65) years, and was diagnosed by ECG and ECHO cardiography.

Group II: hemodialysis with non cardiac complication group: Fifteen patients their ages averaged 41.71±8.84 (rang 26-50) years.

Group III: Control group: Fifteen healthy control subject their ages averaged 37.40±8.58 (rang 26-52) years.

All patients and controls were submitted to the following:

- A-Full history and clinical examination.
- Routine laboratory investigations including :
- -Complete blood counts (CBC) was determined using fully automated cell counter (Sysmex KX-21-N) Japan.

--Liver (ALT, AST, Serum protein, albumin, total Bilirubin and ALP), kidney (blood urea, serum creatinine) function tests , serum calcium and serum phosphorus and lipid profile all of them were estimated on Hitachi 911 autoanalyzer using Rouche reagent kits..

-ECG and Echocardiography.

-ADMA and NO were estimated by ELISA using reagent kits from Immun-diagnostix and G assay designs respectively.

Patients who were diabetics, smokers, who had concurrent infection or who had systemic inflammatory disease as SLE were excluded from the study.

Statistical analysis

The statistical analysis of data was done by using statistical package for social science (SPSS) version 16 on windows XP. The description of data was done as mean ±SD for quantitive data. The analysis of data was done to test statistical significant difference for quantitive data using Student't test. Measuring the mutual correspondence between two values was done using the pearson's correlation coefficient. P value was considered significant if ≤0.05 at confidence interval of 95% (Sokal and James,1995).

3. Results

This study included 30 patients with chronic renal failure(CRF) by impairment of renal functions (blood urea, serum creatinine, creatinine clearance and proteins 24 hours) all patients on hemodialysis three setions/week .They were 25 males (83.3%) and 5 females (16.7%), their ages ranged between (26 to 65 years) with mean±SD (46.73±9.72), their disease duration ranged between 0.33 to 10 years with mean ± SD (3.02±2.90) years.

This study also included 15 apparently healthy individuals as control group. They were 4 males (26.6%) and 11 females (73.3%), their ages ranged between (26 to 52 years) with mean±SD (37.40±8.58).

There was highly significant increase in the mean ±SD of blood urea and serum creatinine (131.40 ± 37.41) & (11.13±2.48….) of patients group compared with the control group (29.33±7.29) & (0.73±0.22) respectively (P<0.001), also there was highly significant increase in the mean ±SD of serum cholesterol and triglyceride (192.43±49.69) &

(189.43±57.62) of patients group compared with control group(143.87±39.05) & (146.07±19.55) respectively (*P*<0.001).(Table 1)

As regard ADMA and NO there was highly significant increase in the mean ±SD of serum ADMA (0.99±0.56) of patients group compared to control group (0.27±0.17) (*P*<0.001). While there was highly significant decrease in mean ±SD of serum NO (7.14±3.59) of patients group compared to control group(13.81±1.57) (*P*<0.001) (Table2, Fig. 1).

There was highly significant increase in the mean ±SD of serum ADMA (1.03 ± 0.58) of cardiac complicated patients group compared to control group (0.27±0.17) (*P*<0.001) while there was highly significant decrease in mean ±SD of serum NO (6.70±3.88) of cardiac complicated patients group compared to control group (13.81±1.57….) (*P*<0.001) (Table 3, Fig2).

There was highly significant increase in the mean ±SD of serum ADMA (0.94 ± 0.56) of non cardiac complicated patients group compared to control group (0.27±0.17) (*P*<0.001) while there was highly significant decrease in mean ±SD of serum NO (7.64±3.31) of non cardiac complicated patients group compared to control group (13.81±1.57….) (*P*<0.001) (Table 4, Fig2).

Asymmetrical dimethylarginine is an endogenous competitive inhibitor of nitric oxide synthase and reduces NO generation which was highly significant increase in cardiac complicated patients group compared with non cardiac complicated patients group (*P*<0.001) while there was highly significant decrease in NO in cardiac complicated patients group compared with non cardiac complicated patients group (*P*<0.001) (Table 5).

There was significant negative correlation between serum ADMA and serum NO, creatinine in patients group (r=-0.645 *p*< 0.05) (r=-0.463 *p* < 0.05) respectively but between ADMA and serum cholesterol there was positive significant correlation(r=0.508 *p* <0.05) while there was insignificant correlation between serum ADMA and blood urea, triglyceride in patients group (r=-0.277 *p* >0.05) (r= 0.322 *p* >0.05) respectively (Table 6).

Table (1) Mean values o f urea, creatinine, cholesterol and triglycerideIn 30 chronic renal failure patients compared to 15 control

Parameter	Patients	Control	***P*** value
Urea (mg/dl)	131.40±37.41	29.33±7.29	<0.001
Creatinine (mg/dl)	11.13±2.48	0.73±0.22	<0.001
Cholesterol(mg/dl)	192.43±49.69	143.87±39.05	<0.001
Triglyceride(mg/dl)	189.43±57.62	146.07±19.55	<0.001

*$p \leq 0.001$ is highly significant

Table (2) Mean values of ADMA and NO in30 chronic renal failure patients compared with15 controls.

Parameter	Patients	Control	***P*** value
ADMA(µmol/l)	0.99±0.56	0.27±0.17	<0.001
NO (µmol/l)	7.14±3.59	13.81±1.57	<0.001

*$p \leq 0.001$ is highly significant

Table (3) Mean values of ADMA and NO in15 patients with cardiac complicated patients compared with 15 controls.

Parameter	Patients	Control	***P*** value
ADMA(µmol/l)	1.03±0.58	0.27±0.17	<0.001
NO (µmol/l)	6.70±3.88	13.81±1.57	<0.001

*$p \leq 0.001$ is highly significant

Table (4) Mean values of ADMA and NO in15 patients without cardiac complication compared with 15 controls.

Parameter	Patients	Control	***P*** value
ADMA(µmol/l)	0.94±0.56	0.27±17	<0.001
NO (µmol/l)	7.64±3.31	13.81±1.57	<0.001

*$p \leq 0.001$ is highly significant

Table (5) Mean value of ADMA and NO in 15 patients with cardiac complication compared with 15 patients without cardiac complication.

Parameter	Patients(cardiac)	Patient(non)	***P*** value
ADMA(µmol/l)	1.03±0.58	0.94±0,56	<0.001
NO (µmol/l)	6.70±3.88	7.64±3.31	<0.001

*p ≤ 0.001 is highly significant

Table (6) correlation between ADMA and NO, creatinine, urea, cholesterol, and triglyceride in 30 patients with chronic renal failure.

Parameter	ADMA r	*P value
NO (µmol/l)	- 0.645	≤0.05
Creatinine(mg/dl)	- 0.463	≤0.05
urea(mg/dl)	- 0.277	>0.05
Cholesterol(mg/dl)	0.508	<0.05
Triglyceride(mg/dl)	0.322	>0.05

*$p \leq 0.05$ is significant

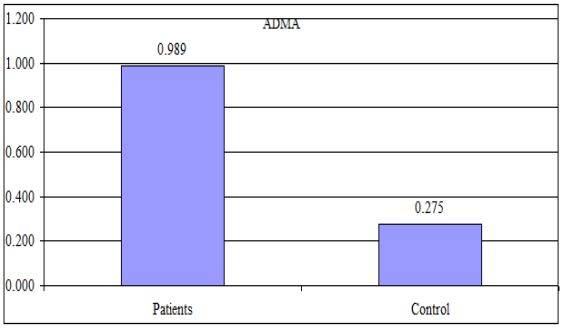

Fig1 Comparison between patients and controls as regard ADMA

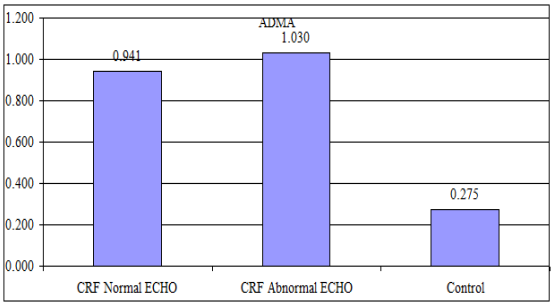

Fig 2 Comparison between groups of patients and control as regard ADMA

4. Discussion

Nitric oxide (NO), a molecule of key importance for the vascular system, is synthesized by endothelial cells. NO is a mediator of immunity and inflammation, and its functions include inhibition of platelet adhesion and endothelial vasodilatation, NO is produced by 3 isoforms of nitric oxide synthase (NOs): type I neuronal (n NOS), type II inducible (I Nos) and type III endothelial (eNOS).(Sahin *et al.,* 2006).

Risk factors for endothelial dysfunction in vascular diseases is due to NO bioavailability. NO has an important effect on regulation of systemic blood pressure and local blood flow, reduced bioavailability of NO is believed to play a role in atherosclerotic vascular damage (Perricone *et al.,* 2005).

Asymmetric dimethylarginine is an endogenous analogue of L –arginine that can modulate NO production, and is an endogenous competitive inhibitor of NO synthase, increased plasma ADMA levels is considered to be a vascular risk factor(Boger *et al.,* 2005) and was first found to be elevated in hemodialysis patients. It has been shown to correlate with traditional and nontraditional cardiovascular risk factors. ADMA is also a strong predictor of cardiovascular events and death in both patients with CKD (stages 2-5) and in the general population. Moreover, ADMA predicts the progression of CKD. (Veldink *et al.,* 2009).

The results of the present study indicate that decreased levels of plasma NO and increased levels of plasma ADMA in patients with CRF than in controls. Elevated ADMA level in the circulation are a combined result of impaired kidney function and a reduced activity of the enzymatic catabolism of ADMA by dimethylarginine dimethylaminohydrolase (DDAH) (Baylis, 2006).

Our findings are consistent with the reports of Sadollah *et al.,* 2010, **Rainer** *et al.,* 2004 and Ravani *et al.,* 2005 who also noted increased plasma ADMA in CKD patients compared with those in patients with normal renal function.

Endothelial dysfunction occurs before the onset of overt vascular disease, suggesting that impaired availability of biologically active nitric oxide, which has an important role in the regulation of renal function in health and disease, (Bogar *et al.,* 2001), contributes to progression of cardiovascular disease(Bogar *et al.,* 1998). The endothelium in patients with end-stage renal disease is dysfunctional, as suggested by findings of several clinical studies, (Hand *et al.,* 1998).

We found that lower plasma level of NO and elevated plasma level of ADMA in CRF patients with cardiac complication vs non cardiac complication. Our results are in agreement with findings of other studies by Carmine *et al.*,2001 , Zoccali *et al.,* 2001 and Ravani *et al.*,2005 who also found that plasma ADMA was a strong and independent cardiovascular

risk factor in chronic renal failure patient. In another study in patients with end-stage renal disease, Zoccali *et al.,* 2002 reported that elevated plasma ADMA concentration was associated with left ventricular dysfunction and left ventricular hypertrophy, important risk factors for mortality in these patients.

In the present study, we found a negative correlation between plasma ADMA and NO level. As nitrosylation caused by NO itself, may diminish DDAH enzyme activity and lead to accumulation of ADMA.

In conclusion, Plasma ADMA level is elevated in the chronic renal failure patients. Also plasma ADMA levels predict cardiovascular events in patients with chronic renal failure.

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