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Level of Asymmetric Dimethylarginine (ADMA) in Children with End Stage Renal Disease (ESRD) on Regular Hemodialysis

KEYWORDS: asymmetric dimethylarginine, dialysis, end stage renal disease, nitric oxide

Abdel Malak CA	Fadel FI	Moussa TE
Chemistry Department, Faculty of Science, Damietta University, Egypt	Pediatrics Department, Faculty of Medicine, Cairo University, Egypt	Biochemistry Department, Genetic Engineering and Biotechnology Division National Research Centre, Cairo, Egypt

Badr AM	Al Ramlawy AM
Pediatrics Department, Faculty of Medicine, Cairo University, Egypt	Chemistry Department, Faculty of Science, Damietta University, Egypt

ABSTRACT *Background: Asymmetric dimethylarginine (ADMA) is an endogenous amino acid similar to L-arginine and able to inhibit the enzyme endothelial nitric oxide synthase (eNOS). It is a factor of impaired nitric oxide (NO) synthesis. Serum levels of ADMA in chronic kidney disease (CKD) increase due to defective inactivation and excretion. Objectives: The aim of the study was to analyze associations between serum ADMA and NO levels and function in children with end stage renal disease (ESRD) on regular hemodialysis (HD). Subjects and methods: The study was included 30 children (17 males, and 13 females) with ESRD on regular hemodialysis aged 11.67±4.47 years, treated with HD for 4.77±2.61 years. Serum ADMA and NO levels and biochemical parameters were measured and echocardiography was performed. Serum ADMA and NO levels and biochemical parameters were also measured in the control group of 30 healthy individuals matched for age. Results: Pre-dialysis ADMA level in children with ESRD was 5.40 ± 0.59 mg/dL and was significantly extremely higher than in control group (1.01 ± 0.41 mg/dL; P <0.0001). Moreover, there was extremely high significant decrease in post-dialysis ADMA level (4.89± 0.40 mg/dL) compared with pre-dialysis ADMA level (5.40 ± 0.59 mg/dL; P < 0.0001) in children with ESRD. On the other hand, mean pre-dialysis level of NO in children with ESRD was 21.00 ± 2.26 mg/dL with extremely high significant decreased compared with control group (39.17 ± 4.13 mg/dL; P < 0.0001). Moreover, the mean pre-dialysis level of NO was 21.00 ± 2.26 mg/dL lower than post-dialysis level of NO with a significant increase (22.04 ± 1.20 mg/dL; P < 0.05). Pre-dialysis ADMA level no correlated with pre-dialysis NO level (r = -0.203; p < 0.282). While, Serum ADMA negative correlated with NO in post-dialysis (r = -0.415; p < 0.022). There were significant positive correlations between Kt/V and ADMA decline (r = 0.344; P < 0.05) and NO increment (r = 0.450; P < 0.01). There was no significant difference (p> 0.05) between males and females in mean pre-dialysis or post-dialysis ADMA levels. Moreover, there was no significant difference (p> 0.05) between males and females in mean pre-dialysis and post-dialysis NO levels. Conclusion: Increased ADMA levels seem to be associated with ESRD on regular hemodialysis. The ADMA levels seem to play an important role in the regulation of the NO production during HD. There was no affect of gender on mean pre-dialysis and post-dialysis levels of ADMA or NO.*

Introduction

Chronic kidney disease is a global public health problem, affecting 10–16% of the adult population worldwide (Levey *et al.*, 2011). Chronic Renal Failure (CRF) is a common renal problem, where the renal injury is of a more sustained nature, which is often not reversible, leading to a progressive destruction of the nephrons and culminating in glomerular and tubular insufficiencies (Klahr *et al.*, 1988). Asymmetric dimethylarginine (ADMA) is formed through the activity of protein-N-methyltransferases which utilizes S-adenosylmethionine as a methyl group donor; an intermediate in homocysteine metabolism (Böger and Zocalli, 2003). Only 20% of ADMA released in urine and about 80% of ADMA cleared by enzymatic degradation of dimethylamine dimethylamino hydrolase (DDAH) (Achan *et al.*, 2003). High ADMA concentrations in patients with end-stage renal disease may contribute to their excess cardiovascular event rate (Böger and Zocalli, 2003). In blood vessel, nitric oxide relaxes vascular smooth muscle to increase blood flow, and suppresses the processes involved in vascular disease including leukocyte adhesion, platelet aggregation, and vascular smooth muscle cell proliferation (Cooke, 2004). NO is also important in vascular regeneration as it mediates angiogenesis (Jacobi *et al.*, 2005) and mobilizes circulating endothelial progenitor cells. Consequently, an endogenous NO inhibitor such as ADMA should be associated with vascular disease (Thum *et al.*, 2005). Proteolysis of proteins containing methylarginine residues leads to the release of ADMA into the plasma (Leiper and Vallance, 1999). Some previous studies have indicated that, accumulation of ADMA is acting as competitive inhibitor for nitric oxide synthase (NOS) (Kielstein *et al.*, 2007). Chronic kidney disease is associated with increased levels of ADMA, which is predictive of increased mortality and cardiovascular disease (Kielstein et al., 2007) whereas concentrations of arginine, the endogenous substrate for NOS, are reduced (Vallance *et al.*, 1992). The aim of this study was to investigate relation of ADMA and NO to ESRD & haemodialysis & to explore associations between ADMA, NO and clinical characteristics of children with ESRD undergoing haemodialysis.

Subjects and Methods

1. Subjects

Thirty children (17 males, and 13 females) with ESRD on regular haemodialysis aged between 4-17 years, with mean age 11.67±4.47 years were attending the Cairo University Center of Pediatric Nephrology and Transplantation (CPNT) were enrolled in the study. The mean duration of haemodialysis of studied patients was 4.77±2.61 years. All patients were clinically stable and free of active infections, diabetes mellitus and autoimmune diseases. They were receiving treatment in the form of erythropoietin and folic acid (for anemia) and antihypertensive drugs (for hypertension if present). None of the patients was receiving antibiotics, corticosteroids or cytotoxic drugs at the time of the study. Studied patients were

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

randomly chosen with different etiologies of their ESRD and were receiving 3 sessions/week. The mean Kt/V of studied patients was 1.85± 0.27, where Kt/V is an universal term used to describe the clearance of urea, K, is the solute clearance across the dialysis membrane, t, the treatment length , and V the volume of urea distribution in the body, generally it used to describe the efficiency of the dialysis process. Thirty healthy children with matched age and sex (16 males and 14 females), with mean age 11.40±4.47 years were taken as control group. Factors that may interfere with the results were excluded including (drugs or inflammatory disorders).

Serum samples
Venous blood samples were obtained in the morning after a 12 h fasting. Serum was separated immediately by low speed centrifugation (4000 rpm for 10 min at 4 °C) and separated to be used freshly for analysis by biochemists those were blinded to classification of subjects as ESRD patients and control.

2. Methods
Kidney Function tests:
Kidney functions were determined spectrophotometrically using an automated analyzer (Bayer Opera biochemical analyzer, Germany). It is an automated chemistry analyzer designed for in vitro diagnostic use in clinical laboratories in Cairo University Center of Pediatric Nephrology and Transplantation (CPNT).

Determination of ADMA levels in human serum
Asymmetric dimethylarginine (ADMA) levels were measured using an ELISA kit (The Kit obtained from GLORY Science Co., Ltd, USA) Using a standard curve, the absorbance of the ADMA-antibody horseradish peroxidase complex in sample was measured at 450 nm. Asymmetric dimethylarginine concentrations of serum samples were determined in mg/dL.

Nitric Oxide Assay
Serum nitric oxide (NO) was estimated by kinetic method using Biodiagnostic kit (Egypt) according to the method described by Montgomery. (1961) (Montgomery *et al.,* 1961)

3. Statistical analysis:
Statistical analysis was performed using a computer-based program (SPSS version 17). The quantitative data are presented as mean ± standard deviation. Unpaired independent t test was used as the statistical test of significance for two groups. A P-value less than 0.05 were considered as statistically significant.

Results
1. Dialysis variables of children with ESRD and control
The mean weight of children with ESRD was significantly lower than control group, 22.50 ± 7.20 Kg versus 33.00 ± 11.70 Kg, respectively ; $p < 0.0001$. Moreover, there was significant decrease in height of children with ESRD (115.97 ± 22.01 cm) compared to control (131.50± 21.45 cm; $p < 0.05$). There was an extremely high significant decrease in eGFR (estimated glomerular filtration rate) in children with ESRD (9.62±2.98 ml/min/m²) compared with control (112.37±21.70 ml/min/m²), $p < 0.0001$. Moreover, A high significant difference was observed between studied children with ESRD and control in the mean arterial pressure (MAP), where mean pre-dialysis MAP in children with ESRD was (88.88±11.18 mmHg) compared with control (80.27±7.81 mmHg), $P < 0.001$; table (1).

2. Biochemical parameters of pre-dialysis children with ESRD and control
An extremely high significant decrease observed in hemoglobin of children with ESRD (9.62±1.71 g%) compared to control (12.76±1.25 g%), with $P < 0.0001$ and the same for hematocrit in children with ESRD (29.72 ± 5.00%) compared to control (39.16 ± 4.66), $P < 0.0001$. On the other hand, there was no significant difference between children with ESRD and control in WBCs and platelets. There was a significant decrease in albumin level of children with ESRD, mean (3.60±0.52 g/dL) compared with control (4.02±0.50g/dL), $P < 0.05$ and there was a significant decrease in calcium level in children with ESRD, where mean was (8.37±1.16 mg/dL) compared with in control (9.11±0.76 mg/dL), $P < 0.05$.

For alkaline phosphatase (ALP), there was an extremely highly significant increase in ALP of children with ESRD, where mean was (530.03±414.19 mg/dL) compared with control (226.50±99.01mg/dL) with $P < 0.0001$. Also, there was an extremely high significant increase in creatinine level of children with ESRD, where mean was (6.46±2.00 mg/dL) compared with control (0.60±0.14 mg/dL) with $P < 0.0001$. The same for blood urea nitrogen (BUN), where mean in children with ESRD was (68.37±21.80 mg/dL), and in control was (13.80±1.71 mg/dL) with $P < 0.0001$. On the other hand, there was no significant difference for phosphorus between children with ESRD and control; table (2).

3. Evaluation of asymmetric dimethylarginine (ADMA) in children with ESRD and control
The mean pre-dialysis ADMA level in children with ESRD was (5.40 ± 0.59 mg/dL) higher than in control (1.01 ± 0.41 mg/dL) with extremely high significant increased ($P < 0.0001$) compared with control group. Moreover, in children with ESRD, the mean pre-dialysis ADMA level was (5.40 ± 0.59 mg/dL) higher than mean post-dialysis level (4.89± 0.40 mg/dL) with extremely high significant decrease ($P < 0.0001$); Figure (1A).

4. Evaluation of nitric oxide (NO) in children with ESRD and control
The mean pre-dialysis NO level in children with ESRD was (21.00 ± 2.26 mg/dL) lower than the mean level in control (39.17 ± 4.13 mg/dL) with highly significant decreased ($P < 0.0001$) in NO level compared with control group. On the other hand for NO in ESRD patients (pre and post dialysis), the mean pre-dialysis NO level was (21.00 ± 2.26 mg/dL) lower than mean post-dialysis NO level (22.04 ± 1.20 mg/dL), with a significant increased ($P < 0.05$) in NO level in post-dialysis compared with pre-dialysis; Figure (1B). And neither post-dialysis ADMA nor post dialysis NO was correlated with post-dialysis MAP.

5. Effect of gender on pre- and post-dialysis ADMA and NO levels
Regarding to gender, the mean pre-dialysis levels of ADMA were (5.30 ± 0.63 mg/dL) in males and (5.52 ± 0.53 mg/dL) in females with no significant difference ($p > 0.05$). And also the mean post-dialysis levels of ADMA were (4.89 ± 0.46 mg/dL) in males and (4.88 ± 0.34 mg/dL) in females with no significant difference ($p > 0.05$); figure (2A). On the other hand, the mean pre-dialysis levels of NO were (21.71 ± 1.84 mg/dL) in males and (21.20 ± 2.77 mg/dL) in females with no significant difference ($p > 0.05$). And also, the mean post-dialysis NO level was (21.80 ± 1.62 mg/dL) in males and was (22.36 ± 2.25 mg/dL) in females with no significant difference ($p > 0.05$); figure (2B).

6. Correlations between ADMA and NO levels in children with ESRD
There was no significant correlation between pre-dialysis ADMA level and pre-dialysis NO level in children with ESRD ($r = -0.203$; $p < 0.282$). While, there was negative significantly correlation between post-dialysis ADMA level and post-dialysis NO level ($r = -0.415$; $p < 0.022$); figure (3). And there were significant positive correlations between Kt/V and ADMA decline ($r = 0.344$; $P < 0.049$); figure (4A). Moreover, there were significant positive correlations between Kt/V and NO increment ($r = 0.450$; $P < 0.013$); figure (4B).

Discussion
Essential goals of dialysis in children are to restore and maintain solute and fluid balance, and maintain life with a reasonable quality, even with the presence of complications of ESRD (Blowey and Alon, 2005). Continuous ambulatory peritoneal

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

dialysis (CAPD) extends the lives of ESRD patients by separating colloids and crystalloids in peritoneal capillary blood using dialysis fluids containing high concentrations of dextrose, acting as an osmotic agent (Lamb *et al.,* 2006). During CAPD, glucose degradation products, low pH and high osmolality increase reactive oxygen species (ROS) production (Tarng *et al.,* 2002). Reactive oxygen species may directly alter proteins with the eventual formation of oxidized amino acids (Miyata *et al.,* 2000). Alternatively, the presence of low pH and renal failure increase protein degradation (Walls, 1997). Proteolysis of proteins containing methylarginine residues leads to the release of ADMA into the plasma (Leiper and Vallance, 1999). Nitric Oxide is identified as endothelium-derived relaxing factor, which help to regulate the blood pressure (Moncada and Higgs, 1993). Decreased estimated glomerular filtration rate (eGFR) and increased urinary albumin excretion predict the major health outcomes of chronic kidney disease, including end-stage renal disease (ESRD) and death, across a wide range of settings (Fox *et al.,* 2012). In this study, children with ESRD showed significantly lower anthropometrical parameters than control in the form of weight which indicate acute malnutrition and height which indicate chronic malnutrition. This agrees with national registries of children with CKD, the mean weight z-score is lower than the American matched mean, and mean height z-scores are below the American age-matched mean (Ho and Stablein, 2003). Also, there was an extremely high significant decrease in eGFR in patients with ESRD compared with control agrees with Stanton, 2013 who reported that in the USA, about 14% of the population has CKD defined as an estimated glomerular filtration (eGFR) of <60 ml/min and/or an increased urine albumin/creatinine ratio of >30 mg/g (Stanton, 2013). Our results indicate to a high significant difference was observed between studied patients and control in MAP agreed with results obtained by Wang *et al.,* 2009, where patients with predialytic MAP <90 mm Hg and patients with an increase of MAP >15 mm Hg during HD sessions were associated with increasing mortality in Kaplan-Meier analysis (p = 0.033 and p = 0.012). Also, there was a highly significant difference between pre- and post- dialysis MAP (p = 0.0001) and this was agreed with the study which was done by El-Sonbaty (El-Sonbaty, 2006).

Anemia is a common complication in HD patients. Additionally, nephrologists frequently witness a rapid and significant drop in their patients' hematocrit during the course of various acute events that regularly take place in this sensitive population (Eleftheriadis *et al.,* 2009). We found that an extremely high significant decrease observed in hemoglobin and hematocrit of patients in comparison with control. Almost universally, patients with ESRD have disturbances in bone and mineral metabolism (Hutchison *et al.,* 1993). Common laboratory manifestations include disturbances in calcium, phosphate, and parathyroid hormone (PTH) concentrations (Levin *et al.,* 2007), in our study it is observed that children on regular dialysis had much lower levels in albumin, calcium levels than control, also they have higher levels of ALP.

In the present work, pre- and post- dialysis ADMA were in children with ESRD significantly higher than control and ADMA level dropped significantly after dialysis. This is in agreement with many studies as (Kielstein *et al.,* 1999; Mochizuki *et al.,* 2005) reported significant lower levels of ADMA in patients treated with peritoneal dialysis. Accumulation of ADMA in ESRD appears to be due in large part to a down-regulated expression of dimethylarginine dimethylamino hydrolase (DDAH), the enzyme which degrades ADMA (Tatematsu *et al.,* 2007). Lack of effectiveness of dialysis to lower ADMA has been in part attributed to protein binding as well as possible redistribution of this molecule during HD (Kielstein *et al.,* 2004). In this context, red blood cells are capable to either buffer or release ADMA (Billecke *et al.,* 2006). Such a mechanism may be influenced by the degree of renal anemia as well as the artificial circulation during dialysis. Kielstein *et al.,* 1999 and Mochizuki *et al.,* 2005 observed no immediate decline of ADMA levels following a regular dialysis session; however 4-5 h after the end of HD a marked decline by 45-50% was observed. Increase of dialysis dose and frequency was also shown to be ineffective to lower ADMA levels (Chan *et al.,* 2005).

It has been demonstrated that ADMA significantly inhibits NO formation when present in blood at a concentration range of 3–15 uM (Kurose *et al.,* 1995; Bergamini *et al.,* 2004). In our study we found that pre- and post- dialysis NO level were in children with ESRD significantly lower than control and there was highly significant increment in NO level after dialysis and neither post-dialysis ADMA nor post dialysis NO was correlated with post-dialysis MAP, this is in contrast to Engelberger *et al.,* 2009 who found that there was no acute influence of HD on an NO-dependent vasodilatory response in the skin microcirculation. Bergamini *et al.,* (2004) who studied the relation of ADMA and NO to blood pressure of patients on regular hemodialysis before, during and at the end of haemodialysis. The HD procedure significantly removes ADMA from plasma of stable-HD patients, while in the hypotension-prone ADMA levels are unchanged at the end of the HD. Moreover, in the hypotension-prone patients, during the hypotensive episode, a dramatic drop of ADMA levels is observed, followed by a rapid increase at the end of the HD. These findings published by Bergamini *et al.,* (2004) are not coinciding with our study. In the current study we measured ADMA and NO at beginning and end of dialysis session only. There was no significant correlation between pre-dialysis ADMA and pre-dialysis NO. Also it was found that post-dialysis ADMA significantly negative correlated with post dialysis NO. It has been demonstrated that ADMA significantly inhibits NO formation when present in blood at a concentration range of 3–15 uM (Kang *et al.,* 1999; Bergamini *et al.,* 2004).

In our study there was no significant difference between males and females as regard either ADMA decline or NO increments, this may be due to our patient's age from 4-17 years, where most of them still in childhood period, so influence of sex hormones is minimal. On the other hand, Valtonen *et al.,* (2010) reported that the circulating ADMA concentration varies across the menstrual cycle in young women ADMA (P=0.017), L-arginine (P=0.002), and ADMA/SDMA ratio (P<0.001) were significantly lower in the luteal phase than in the follicular phase of the menstrualcycle, this indicates that sex hormones influence ADMA level.

Table (1): Demographic data of ESRD patients and control

Variables		Patients [a]	Control	P Value [e]
Total number		30	30	-
Gender	Male, n (%)	17 (56.7%)	18 (60.0%)	> 0.05
	Female, n (%)	13 (43.3%)	12 (40.0%)	> 0.05
Age (yrs), mean ± SD [b]		11.67 ± 4.47	11.40 ± 4.47	> 0.05
Weight (Kg)		22.50 ± 7.20	33.00 ± 11.70	< 0.0001***
Height (cm)		114.83 ± 24.68	131.50 ± 21.45	< 0.05*
Duration of dialysis (yrs) , mean ± SD		4.77 ± 2.61	----	----
Kt/V, mean ± SD		1.85 ± 0.27	----	----
eGFR (ml/min/m^2) [c], mean ± SD		9.62 ± 2.98	112.37 ± 21.70	< 0.0001***
MAP (mmHg) [d] : Pre-dialysis		88.88 ± 11.18	80.27 ± 7.81	< 0.001**
Post-dialysis		82.88 ± 8.60	----	----

[a] Patients: Patients with End Stage Renal Disease under regular hemodialysis.

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

[b] **SD: Standard deviation.**

[c] **eGFR : estimated glomerular filtration rate, which calculated from**

Calculated GFR (ml/min) =

$$\frac{[140 - \text{age (years)}] \times \text{weight (kg)} \times 1.23 \ \text{(males only)}}{\text{Serum creatinine (micromole/L)}}$$

[d] MAP: mean arterial pressure and MAP (mmHg) = [(2 x diastolic)+systolic] / 3

[e] P Value: p > 0.05 non-significant; *p < 0.05 significant; **P < 0.001 high significant and ***P < 0.0001 extremely significant.

Table (2): Pre-dialysis variables of patients and control

Pre-dialysis variables	Patients [a] (n=30)	Control (n=30)	P Value [c]
	Mean ± SD	Mean ± SD	
Hemoglobin (g %)	9.62 ±1.71	12.76 ± 1.25	< 0.0001***
Hematocrit (%)	29.72 ± 5.00	39.16 ± 4.66	< 0.0001***
WBCs (x1000/ mm^3)	6.72 ± 1.95	6.72 ± 1.96	> 0.05
Platelets (x1000/ mm^3)	232.77 ± 74.26	245.67 ± 70.34	> 0.05
Albumin (g/dL)	3.60 ±0.52	4.02 ± 0.50	< 0.05*
Calcium (mg/ dL)	8.37 ± 1.16	9.11 ± 0.76	< 0.05*
Phosphorus (mg/ dL)	4.60 ±1.28	4.43 ± 0.63	> 0.05
ALP (mg/ dL)	530.03 ±414.19	226.50 ± 99.01	< 0.0001***
BUN (mg/ dL)	68.37 ± 21.80	13.80 ± 1.71	< 0.0001***
Creatinine (mg/ dL)	6.46 ± 2.00	0.60 ± 0.14	< 0.0001***

[a] Patients: **Patients with End Stage Renal Disease under regular hemodialysis**, ALP: **alkaline phosphatase; and** BUN: **blood urea nitrogen.**

***Significant, ** high significant and ***extremely high significant.**

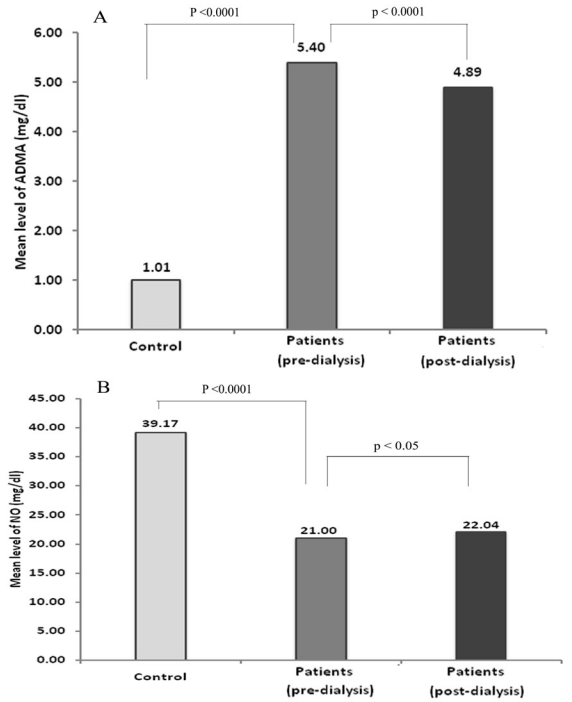

Figure (1): (A) Mean level of ADMA (mg/dl) in patients and control. There was extremely highly significant increased (P < 0.0001) in ADMA level in pre-dialysis patients compared with control and there was an extremely high significant decrease (P < 0.0001) in ADMA level in post-dialysis patients compared with pre-dialysis patients. (B) Mean level of NO (mg/dL) in patients and control. There was extremely high significant difference (P < 0.0001) between NO level in patients at pre-dialysis compared with control and there was a significant increased (P < 0.05) in NO level in post-dialysis patients compared with pre-dialysis patients.

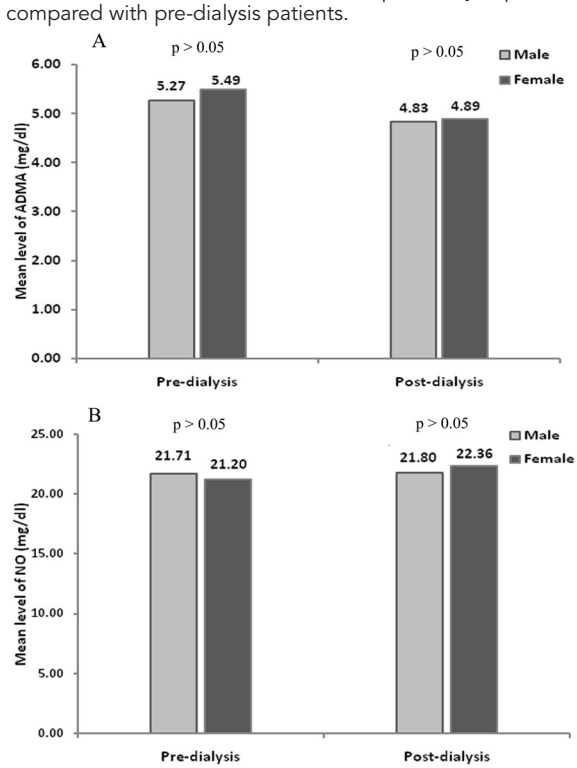

Figure (2): (A) Mean level of ADMA (mg/dL) according to gender. There was no significant difference (P > 0.05) in

Volume : 4 | Issue : 2 | Feb 2014 | ISSN - 2249-555X

pre- or post-dialysis ADMA between males and females. (B) Mean levels of NO (mg/dL) according to gender. There was no significant difference (P > 0.05) in pre- or post-dialysis NO between males and females.

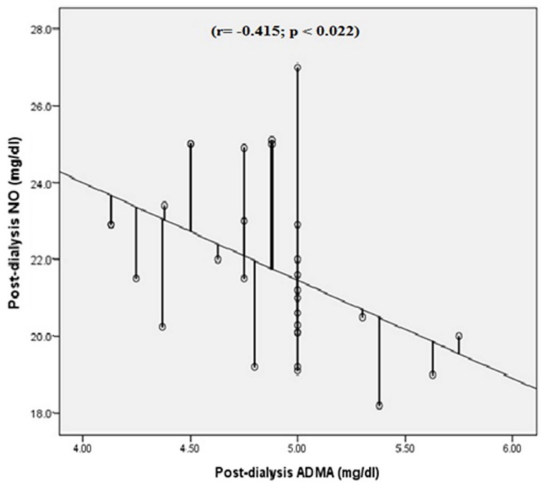

Figure (3): Correlation between post-dialysis ADMA and NO.

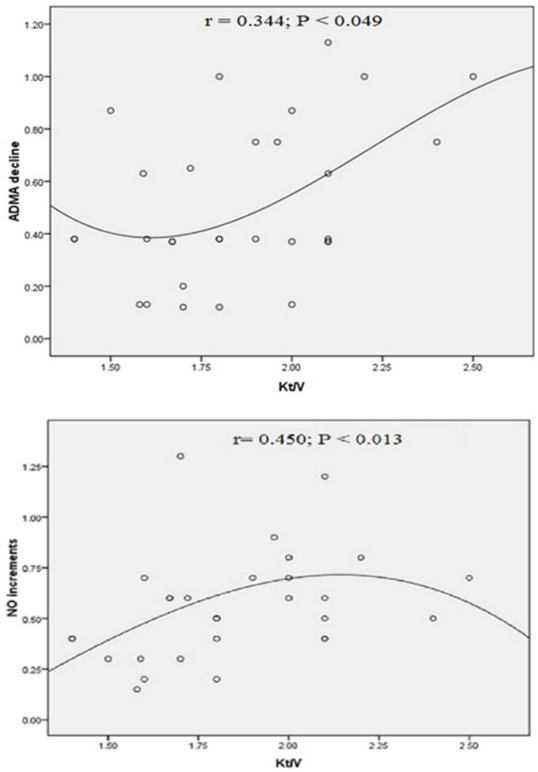

Figure (4): (A) Correlation between ADMA decline and Kt/V. (B) Correlation between NO increment and Kt/V.

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