ASYMMETRIC DIMETHYLARGININE SERUM LEVELS ARE ASSOCIATED WITH PATIENT CHARACTERISTICS AFTER RENAL TRANSPLANT

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Summary

Objectives: To investigate the changes in asymmetric plasma dimethylarginine (ADMA) levels after six months of renal transplant and their potential associations with patient characteristics. ***Subjects and methods:*** From March 2018 to April 2020, a prospective enrolled 75 recipients 6 months after renal transplant at Military Hospital 103. ***Results:*** The average ADMA concentration at 6th month of the transplant was 0.57 µmol/L, a statistically significant decrease from the time before transplant (0.62 µmol/L) with $p < 0.05$, but still higher than the healthy controls (0.17 µmol/L), $p < 0.001$. At 6th month following the transplant, 32.0% of patients had an increase and 68.0% had a decrease in ADMA concentration. After transplant, increased ADMA concentration was found to be positively correlated with the atherogenesis index (r = 0,468) and negatively correlated with the glomerular filtration rate ($r = -0.261$, $p < 0.05$). ***Conclusion:*** Six months after renal transplant, plasma ADMA decreased significantly but remained higher than in healthy people. Following the transplant, both the atherogenesis index and the glomerular filtration rate were significantly associated with an increase in ADMA levels.

** Keywords: Asymmetric Dimethylarginine; Chronic kidney disease; Kidney transplant; Glomerular filtration rate.*

INTRODUCTION

Chronic kidney disease is associated with hypertension, and diabetes is a global health problem that burdens the health sector in many countries [1]. When kidney disease progresses to the end stage, renal replacement therapy is required, with renal transplant considered the best option because it allows patients to live a nearly normal life.

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However, renal transplant recipients continue to face numerous complications, particularly cardiovascular complications such as coronary artery disease and heart failure, and diabetes then appears [2]. Cardiovascular complications persisted in the pre-transplant patient and were influenced by the use of antirejection drugs. Asymmetric dimethylarginine (AMDA) is a nitrite oxide (NO) inhibitor that causes vasoconstriction and atherosclerosis. As a result, in patients with chronic kidney disease, ADMA may be a risk factor for premature death and cardiovascular disease [3]. Recent research has found no consistent change in ADMA levels in renal transplant recipients. Previous research found that ADMA levels decreased after renal transplant [4], while other research found that ADMA levels were significantly higher and correlated with graft rejection [2]. A study on ADMA in patients with end-stage CKD was conducted in Vietnam, but no study on renal transplant recipients was conducted. Therefore, we conducted this study: *To investigate of changes in plasma AMDA levels and the association with the patient's characteristics after six months of a kidney transplant*.

SUBJECTS AND METHODS

1. Subjects

The present study consisted of 2 groups: 75 patients with end-stage chronic kidney disease caused by chronic glomerulonephritis and chronic pyelonephritis who received kidney transplants were followed up on at the time of transplant and six months later.

* *Inclusion criteria*:

Patients aged ≥ 18 were chosen, transplanted and followed up on for six months at Military Hospital 103. All patients were required to sign consent forms.

* *Exclusion criteria:*

We excluded post-transplant recipients who had a decline in kidney function, graft rejection, lacked clinical records, or refused to participate in the study.

2. Methods

* *Study design:* A prospective, cross-sectional description of two-time points.

* *Study procedure:*

- Create a clinical record form.

- Gather data for pre-transplant research indexes.

- Gather data for post-transplant research indexes.

* *Clinical examination method:*

- Patients were interviewed and examined to detect clinical symptoms.

- Hematology, blood chemistry, and urine biochemistry are examples of laboratory tests. Atherogenic index calculation: Calculate automatically based on plasma triglyceride and HDL-C concentrations using the following website: http://www.biomed.cas.cz/fgu/aip/calculator.php

- Using and developing statistics on cardiovascular risk factors like hypertension (as defined by the Vietnam Heart Association in 2015), diabetes (as defined by the American Diabetes Association in 2010), and dyslipidemia (as defined by the Vietnam Heart Association in 2008). Tobacco use, dyslipidemia (Vietnam Heart Association),

* *Test method:*

- Collect hematology and blood biochemistry test results (glucose, urea, creatinine, uric acid, 4 blood lipid indexes, hs-CRP) at two-time points before and six months after transplant.

- Quantification of plasma ADMA concentrations in control and patient groups (two time points: Before transplant and 6 months after transplant): Using ELISA method, Immunndiagnostik AG kit (ADMA ELISA kit, Germany) on biochemistry machine Immuno Diagnostic Automation, Inc; Model ELX800DA, at the Military Medical University's Department of Pathophysiology.

- Estimate the glomerular filtration rate of the patient six months after transplant.

* *Statistical analysis:* SPSS 22.0. Continuous variables are represented by the mean and standard deviation ($\overline{X}$ ± SD) as well as percentages (%). Compare quantitative variables with normal distribution using the T-test and non-normal distribution using the Mann-Whitney and Kruskal-Wallis tests. Comparisons of proportions using the χ^2 test and the Exact Fisher test. Analysis of logistic regression.

RESULTS AND DISCUSSION

At six months after transplant, the average age of the patients was 37.17 ± 9.95 years old, while the control group was 36.82 ± 7.53 years old ($p > 0.05$), with 69.3% of males in the patient group and 62.5% in the control group ($p > 0.05$).

Table 1: Comparison of plasma ADMA concentrations before and six months after transplant.

ADMA	Before Transplant (1) (n = 75)	After Transplant (2) (n = 75)	Control (3) (n = 80)	p
ADMA (µmol/L) Median (quartile)	0.62 (0.49 - 0.74)	0.57 (0.47 - 0.65)	0.17 (0.13 - 0.23)	$p < 0.001^a$ $p(1)(2) < 0.05^b$ $p(1)(3) < 0.001^b$ $p(2)(3) < 0.001^b$
Min	0.27	0.12	0.09	
Max	1.16	1.00	0.37	
BMI	20.76 ± 2.44	21.03 ± 2.51		p(1)(2) > 0.05
Ure	22.1 (17.16 - 30.06)	5.67 (4.66 - 6.8)	4.85 (4.16 - 5.75)	p(1)(2) < 0.001
Creatinin	876.5 (680 - 1079.2)	101.5 (84.9 - 119.2)	81.19 (71.75 - 92.04)	p(1)(2) < 0.001
Acid uric	428 (327 - 535)	382.4 (336.8 - 453)	333.37 (284.33 - 387.67)	p(1)(2) < 0.05
Cholesterol	4.05 ± 1.10	4.74 ± 1.20	4.58 ± 0.50	p(1)(2) < 0.001
Triglycerid	1.57 (0.97 - 2.43)	2,13 (1.44 - 2.88)	1,19 (0.87 - 1.74)	p(1)(2) < 0.01
LDL-C	2.59 ± 0.83	3.13 ± 0.90	2.93 ± 0.45	p(1)(2) < 0.001
AIP	0.17 (-0.06 - 0.41)	0.23 (0.07 - 0.48)		p > 0.05
Hemoglobin	102.09 ± 16.98	134.76 ± 14.29	142.95 ± 12.10	p(1)(2) < 0.001
Dd	50.89 ± 6.28	46.97 ± 5.04		p < 0.001
Left atrium	32.62 ± 5.56	29.84 ± 3.10		p < 0.001

[a]Kruskal Wallis test; [b]Mann-Whitney U test

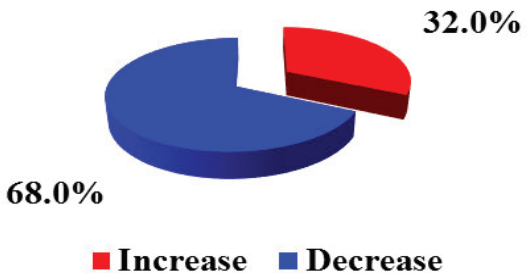

Chart 1: The proportion of patients with changes in ADMA concentration before and after transplant.

The median ADMA concentration after six months of kidney transplant decreased more than before the transplant but remained statistically significantly higher than the healthy group, $p < 0.001$. The results of our study revealed that the plasma ADMA concentration of patients 6 months after renal transplant was 0.57 µmol/L, a statistically significant decrease from the time before transplant (0.62 µmol/L), with $p < 0.05$. Our findings are similar to those of Kathleen J. Claes et al., who followed 167 patients three and twelve months after renal transplant and found that the average ADMA concentration decreased from 0.63 µmol/L (before transplant) to 0.60 µmol/L (3 months after transplant) and 0.55 µmol/L (12 months after transplant) [4].

Biochemical indicators (urea, creatinine, uric acid, cholesterol, LDL-C), hematology (hemoglobin), API, and heart atrial diameter were significantly improved after transplant compared to before transplant; the difference is statistically significant ($p < 0.05$). (0.001). Patients' BMI after transplant, on the other hand, was higher than before, but it is not statistically significant.

ADMA concentration decreased in 68.0% of patients compared to pre-transplant, while it increased in nearly one-third of patients (32.0%) post-transplant. Many studies also show that ADMA levels rise transiently in the early stages of surgery and then fall gradually; however, ADMA levels in post-transplant patients remain higher than in the general population [4]. Some patients had an increase in ADMA levels after transplant, which could explain why some patients had unstable kidney function after six months of transplant, and some anti-rejection drugs such as Inhibition of calcineurin cause loss of homeostasis, glucocorticoids cause an increase in ADMA levels [6]. A higher post-transplant ADMA level than pre-transplant is suggested a dismal prognosis. Higher ADMA concentrations before transplant were found to be significantly associated with an increased risk (HR = 1.43) in Aleksandar's study [7].

Table 2: The correlation between plasma ADMA concentrations and several hematological and biochemical indices at 6th month after transplant.

Index	ADMA (µmol/L)		Correlation equation
	r	p	
Hemoglobin (g/L)	0.157	> 0.05	-
Ure (mmol/L)	0.141		-
eGFR (mL/min)	-0.261	< 0.05	ADMA = 0.773 - 0.003*MLCT
Acid uric (µmol/L)	0.287		ADMA = 0.001*Acid uric + 0.379
Cholesterol (mmol/L)	0.044	> 0.05	-
LDL-C (mmol/L)	0.087		-
AIP	0.468	< 0.001	ADMA = 0.275*AIP + 0.482
Hs-CRP (mg/L)	0.099	> 0.05	-

ADMA is primarily eliminated by the DDHA enzyme and renal clearance. Most kidney transplants function well after transplant, as evidenced by increased eGFR and significantly lower urea, creatinine, and uric acid indexes. As a result of a well-functioning transplanted kidney, ADMA clearance will be realized and gradually reduced. According to research, an increase in ADMA concentration is proportional to a decrease in eGFR [2, 3]. Our findings are consistent with other studies around the world in which ADMA is moderately negatively correlated with eGFR and positively correlated with uric acid. Our research found a positive, average correlation between ADMA and the atherogenesis index, r = 0.468, p < 0.001. A decrease or loss of kidney function in CKD patients causes an accumulation of components in blood lipids and promotes the formation of atherosclerosis, causing disturbances in blood lipid metabolism. However, dyslipidemia persists after transplant, due in part to the use of anti-rejection drugs and in part to the diet and weight gain. As a result, it is critical to maintaining weight control after transplant; gentle exercise will prevent weight gain, reduce obesity and insulin resistance, lower the risk of lipid disorders, and thus lower the risk of cardiovascular events [8].

Table 3: Comparison of patient characteristics according to changes in ADMA concentration 6 months after transplant (n = 75).

Characteristics	Increase (n = 24)	Decrease (n = 51)	p
Hypertention, n (%)	13 (54.2)	32 (64.0)	> 0.05c
Poor BP control, n (%)	5 (20.8)	15 (29.4)	
Diabetes, n (%)	3 (12.5)	0 (0)	
BMI, ($\overline{X}$ ± SD)	20.64 ± 2.79	21.21 ± 2.37	> 0.05a
Dyslipidemia, n (%)	20 (83.3)	45 (88.2)	> 0.05d
Left ventricular hypertrophy, n (%)	7 (29.2)	11 (22.9)	> 0.05c
Hemoglobin (g/L), ($\overline{X}$ ± SD)	130.17 ± 13.18	136.90 ± 14.26	> 0.05s
Anemia, n (%)	9 (37.5)	13 (25.5)	> 0.05c
Ure (mmol/L), ($\overline{X}$ ± SD)	5.69 ± 1.88	5.73 ± 1.51	> 0.05a
Creatinine (µmol/L), ($\overline{X}$ ± SD)	106.2 ± 25.88	100.76 ± 21.64	
eGFR (mL/min), Median (quartile)	72.0 (64.25 - 90.75)	72.0 (66.0 - 85.0)	> 0.05b
Acid uric (µmol/L), Median (quartile)	408.7 (313.5 - 445.80)	380.70 (336.80 - 461.10)	
Cholesterol (mmol/L), ($\overline{X}$ ± SD)	4.60 ± 1.27	4.81 ± 1.17	> 0.05a
LDL-C (mmol/L), ($\overline{X}$ ± SD)	3.01 ± 0.91	3.19 ± 0.90	
AIP, Median (quartile)	0.25 (0.13 - 0.54)	0.21 (0.01 - 0.44)	> 0.05b
Hs-CRP (mg/L), Median (quartile)	0.74 (0.45 - 2.47)	0.78 (0.32 - 1.47)	
Dd (mm), ($\overline{X}$ ± SD)	46.96 ± 4.75	46.79 ± 5.33	> 0.05a
Ds (mm), ($\overline{X}$ ± SD)	28.63 ± 3.24	28.54 ± 4.02	
Left atrium diameter (mm), ($\overline{X}$ ± SD)	29.38 ± 3.60	29.98 ± 2.88	
EF%, ($\overline{X}$ ± SD)	69.08 ± 3.56	69.31 ± 4.64	
LVMI, Median (quartile)	95.85 (78.27 - 112.77)	94.30 (75.80 - 108.05)	> 0.05b

[a]student T test; [b]Mann-Whitney U test; [c]Chi-square test; [d]Fisher's exact test

There was no significant difference in most of the indexes when comparing the clinical and subclinical indexes at T0 (before transplant) in the groups of increasing and decreasing ADMA after 6 months of transplant, $p > 0.05$. However, three patients with post-transplant diabetes were among those with increased post-transplant ADMA. Adipose tissue secretes many hormones and cytokines in patients with chronic kidney disease after kidney transplant, which are thought to be the main factors contributing to fat-associated insulin resistance and endothelial dysfunction, which is part of the cause of post-transplant diabetes. This contributes to the high prevalence of cardiovascular disease in this patient population.

Table 4: Logistic multivariate analysis of factors associated with increased ADMA after 6 months of transplant.

Factors	**Odds ratio (OR)**	**Confidence interval 95%**	**p**
Poor BP control	0.13	0.02 - 0.81	< 0.05
Hemoglobin	0.92	0.86 - 0.98	
Creatinine	1.08	1.01 - 1.16	
eGFR	1.08	0.99 - 1.17	> 0.05
Acid uric	0.98	0.97 - 0.99	< 0.05
Cholesterol	15.65	1.84 - 133.09	
LDL-C	0.12	0.01 - 1.17	> 0.05
HDL-C	0.001	0 - 0.05	< 0.005
Dd	0.75	0.59 - 0.96	< 0.05
LVMI	1.03	0.99 - 1.08	> 0.05
Dyslipidemia	0.12	0.01 - 1.10	

Among 75 patients with adequate post-transplant follow-up, patients with poor blood pressure control had a lower rate and plasma ADMA concentration than the group with controlled blood pressure (OR = 0.13). Our findings are not consistent with those of other studies conducted around the world. Because of the study's small sample size and the fact that the patient's blood pressure was only taken once, the difference is not indicative of the patient's average blood pressure. Sadollah Abedini followed 1847 kidney transplant patients in Norway and discovered that the number of patients with hypertension after kidney transplant was proportional to plasma ADMA concentrations of 0.7 - 0.77 µmol/L, 0.78 - 0.85 µmol/L, and 0.86 µmol/L compared to 0.69 µmol/L [9]. In Anne-Roos S. Frenay's study, which followed 686 kidney transplant recipients, the proportion of people with hypertension was also proportional to the plasma ADMA concentration [3]. Our findings in terms of hemoglobin concentration show that lower post-transplant hemoglobin concentrations will increase ADMA levels. Because red blood cells play an important role in ADMA storage, the lysis of red blood cells releases a large amount of free ADMA, promoting methylated protein degradation [10]. Anemia is caused by a decrease in Erythropoietin production by the kidneys, nutritional deficiencies, blood loss during filtration, inflammation, and metabolic disorders in patients with chronic kidney disease. After the transplant, the above issues were resolved, and the hemoglobin returned to normal; however, there were still some cases of anemia due to blood loss during surgery and viral infection. There is a correlation between glomerular filtration rate and increased ADMA concentration, but not statistically significant.

CONCLUSION

The plasma ADMA concentration 6th month after transplant was 0.57. Increased ADMA concentration was positively related to atherogenesis index and negatively related to glomerular filtration rate after transplant, $p < 0.05$. Hemoglobin, serum creatinine, cholesterol, and HDL-C are all independent factors associated with higher plasma ADMA levels six months after kidney transplant, $p < 0.05$.

REFERENCES

1. Collaboration, G.B.D.C.K.D. (2020). Global, regional, and national burden of chronic kidney disease, 1990-2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet*; 395(10225): 709-733.

2. Pihlstrom, H., et al. (2014). Symmetric dimethylarginine as predictor of graft loss and all-cause mortality in renal transplant recipients. *Transplantation*; 98(11): 1219-1225.

3. Frenay, A.R., et al. (2015). Plasma ADMA associates with all-cause mortality in renal transplant recipients. *Amino Acids*; 47(9): 1941-1949.

4. Claes, K.J., et al. (2014). Time course of asymmetric dimethylarginine and symmetric dimethylarginine levels after successful renal transplantation. *Nephrol Dial Transplant;* 29(10): 1965-1972.

5. Said, M.Y., et al. (2019). Effect of renal function on homeostasis of asymmetric dimethylarginine (ADMA): Studies in donors and recipients of renal transplants. *Amino Acids*; 51(3): 565-575.

6. Graff, J., et al. (2002). Immunosuppressive therapy regimen and platelet activation in renal transplant patients. *Clin Pharmacol Ther*; 72(4): 411-418.

7. Radujkovic, A., et al. (2019). Asymmetric dimethylarginine serum levels are associated with early mortality after allogeneic stem cell transplantation. *Haematologica;* 104(4): 827-834.

8. Teplan, V., et al. (2014). Early exercise training after renal transplantation and asymmetric dimethylarginine: The effect of obesity. *Kidney Blood Press Res*; 39(4): 289-298.

9. Abedini, S., et al. (2010). Asymmetrical dimethylarginine is associated with renal and cardiovascular outcomes and all-cause mortality in renal transplant recipients. Kidney Int; 77(1): 44-50.

10. Davids, M., et al. (2012). Role of the human erythrocyte in generation and storage of asymmetric dimethylarginine. *Am J Physiol Heart Circ Physiol*; 302(8): H1762-H1770.