CHANGES OF PLASMA ASYMMETRIC DIMETHYLARGININE LEVELS IN THE FIRST SIX MONTHS AFTER RENAL TRANSPLANT

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SUMMARY

Objectives: To assess the changes of plasma asymmetric dimethylarginine (ADMA) levels and its potential associations with patient characteristics after six months of renal transplant. ***Subjects and methods:*** A prospective, descriptive study which enrolled 83 recipients at 6 months after renal transplant compared to 83 healthy people at Military Hospital 103 from March 2018 to April 2020. ***Results:*** The plasma ADMA was normally distributed and had a mean value of 0.49 µmol/L at 6 months after renal transplant, it was significantly lower than the level observed before transplant (0.68 µmol/L), but it showed a higher ADMA level than the healthy people (0.32 µmol/L), $p < 0.001$. The change of ADMA levels was 0.17 µmol/L, of which 32.5% of patients with high ADMA levels showed a dramatic decrease to normal, and 9.6% of patients with normal ADMA levels revealed a significant increase in the first six months after renal transplantation. Hemoglobin and hs-CRP levels, hypertension, and dyslipidemia (especially elevated triglyceride) after transplantation are independent determinants associated with an increase in plasma ADMA at 6 months after renal transplantation. ***Conclusion:*** Plasma ADMA levels at 6 months after renal transplantations were remarkably decreased over time but still higher than in healthy people. Elevation of ADMA levels after transplantation was associated with higher hs-CRP, lower hemoglobin, hypertension, and post-transplant dyslipidemia.

* *Keywords:* Plasma asymmetric dimethylarginine; End-stage chronic kidney disease; Renal transplantation.

INTRODUCTION

The increasing rate of chronic kidney disease is associated with hypertension, atherosclerosis, diabetes and is a major clinical problem [1]. When kidney disease progresses to the end-stage, renal replacement therapy is needed. A renal transplant is considered the most appropriate option because it helps patients return to an almost normal life. However, renal transplant recipients still have many issues, especially cardiovascular complications such as coronary artery disease, heart failure [2]. Asymmetric dimethylarginine (AMDA) is an inhibitor of the synthesis of nitrite oxide (NO), which causes vasoconstriction and atherosclerosis. Therefore, ADMA may be a risk factor for premature death and cardiovascular disease in patients with chronic kidney disease [3].

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Recent studies did not show the consistent change of ADMA levels in renal transplant recipients. The previous studies showed that ADMA levels decreased after renal transplant [4], and other studies reported that ADMA levels were significantly high and correlated with graft rejection [2]. In Vietnam, there has been a study on ADMA in patients with end-stage CKD, but no study on renal transplant recipients. Therefore, this study was conducted: *To assess the changes of plasma ADMA levels in the first six months after renal transplant (Tx).*

SUBJECTS AND METHODS

1. Subjects

The present study consisted of 2 groups: 83 renal transplant recipients (RTR) with six months follow-up and 83 healthy people.

* *Inclusion criteria:*

- End-stage CKD group: Patients aged ≥ 18 years were selected, transplanted, and followed up for 6 months at Military Hospital 103. The consent forms were obtained from all patients in order to participate in the study.

- Control group: people without any clinical diseases, same age, and gender distribution as in the study group. The consent forms were also obtained from all control subjects to participate in the study.

** Exclusion criteria:*

- End-stage CKD group: we excluded post-transplant recipients who had a slope of kidney function, graft rejection, and did not have enough clinical records or did not want to participate in the study.

2. Methods

** Study design:* A prospective, descriptive and comparative case-control study.

** Study procedure:*

- If all pre-transplant inclusion criteria were fulfilled, then subjects were enrolled in the study. All patients and controls were explained about the risk and benefits of the study and signed a consent form. Blood pressure was measured according to the guidelines. Bodyweight, height, and BMI were calculated. Patients were recommended to prepare early morning blood collection.

- The results on hematology, blood biochemistry (glucose, urea, creatinine, uric acid, 4 blood lipid indexes, hs-CRP) were collected at two-time points, before Tx and at 6 months after Tx.

- Plasma ADMA measurements were performed in both healthy people and end-stage CKD (Blood samples were taken immediately at two-time points: before transplantation and at 6 months after transplantation): Plasma ADMA was measured by a validated ELISA kit (Immunndiagnostik AG kit, Germany) using an Immuno Diagnostic Automation, Inc; Model ELX800DA, at the Department of Pathophysiology, Military Medical Academy.

- Estimated glomerular filtration rate (eGFR) was calculated at 6 months after Tx.

- Similar procedures were repeated after six months after renal transplantation.

** Statistical analysis:* Statistical analyses were performed using SPSS 22.0 (SPSS Corp. Chicago, IL, USA). Continuous variables were presented as mean, standard deviation ($\bar{X}$ ± SD), and percentages (%). The quantitative variables were compared to a normal distribution by T-test and non-normally distributed parameters using the Mann-Whitney and Kruskal-Wallis test. The paired differences between proportions were performed using the χ^2 test and the Exact Fisher test.

RESULTS AND DISCUSSION

The patient cohort of 83 RTR had a mean age of 38.23 ± 11.21 years, and 69.9% were male, the healthy people had a mean age of 36.61 ± 7.48 years, and 62.7% were male (p > 0.05).

Table 1: Comparison of plasma ADMA levels before and at 6 months after Tx.

ADMA	Before Tx (n = 83)	After Tx (n = 83)	Control (n = 83)	p
ADMA (µmol/L) Median Quartiles)	0.677 (0.497 - 0.785)	0.494 (0.248 - 0.624)	0.319 (0.239 - 0.373)	p(1)(2)(3) < 0.001
Min	0.181	0.124	0.112	p(1)(2) < 0.001
Max	1.167	0.964	0.448	p(1)(2) < 0.001

Plasma ADMA had a mean value at 6 months after Tx was lower than before Tx, but still higher than the healthy group with statistical significance, p<0.001. Table 1 shows that the plasma ADMA levels at 6 months after Tx was 0.49 µmol/L, a statistically significant decrease compared to the time point before Tx (0.68 µmol/L) with p < 0.001. Plasma ADMA levels remained unchanged in 48 recipients (57.8%), 27 out of 83 recipients (32.5%) had decreased ADMA levels compared to before Tx, only 8 recipients (9.6%) showed ADMA levels increased after Tx. ADMA levels observed in the present cohort in agreement with Kathleen J. Claes et al., which followed 167 patients at months 3 and 12 after Tx, the mean value of plasma ADMA decreased from 0.63 µmol/L (before Tx) to 0.60 µmol/L (months 3 after Tx) and 0.55 µmol/L (months 12 after Tx) [4].

Table 2: Distribution of plasma ADMA levels before and at 6 months after Tx.

ADMA	Recipients (n)	Percentage (%)
High to normal	27	32.5
Unchange	48	57.8
Normal to high	8	9.6
ADMA variation, (µmol/L) Median (Quartiles)	0.17 (0 - 0.32)	

In comparison with healthy individuals, plasma ADMA levels overall decreased from high to normal was observed in 32.5% of recipients. The mean change in ADMA at 6 months after Tx was 0.17 µmol/L. ADMA levels are mainly eliminated by the DDHA enzyme and renal clearance. After Tx, parallel to the improvement of renal function, we revealed an increase in eGFR and a marked reduction in urea and creatinine indexes. Thus, a well-functioning transplanted renal means that ADMA clearance will be realized and gradually reduced. Frenay A.R. et al. and Graff J. et al. studies had shown that an increase in ADMA levels is proportional to a decrease in eGFR [2, 3]. Several recipients in the present cohort had an increase in ADMA levels after Tx, which is at least partly explained that the period time at 6 months after Tx, the portion of recipients who had unstable kidney function and immunosuppressive agents such as inhibition of calcineurin cause loss of homeostasis, glucocorticoids cause an increase in ADMA levels [5]. Claes K.J. et al. also reported that ADMA levels tend to increase transiently in the early stages of engraftment and thereafter gradually decrease. However, plasma ADMA levels after transplant were still higher than in healthy people [4].

Table 3: Comparison of recipient characteristics at six months after transplant by the changes of plasma ADMA levels.

ADMA	Decrease (n = 27)	Unchange (n = 48)	Increase (n = 8)	P
Hypertention, n (%)	17 (65.4)	29 (60.4)	4 (50)	> 0,05 [e]
Diabetes, n (%)	2 (7.4)	6 (12.5)	0 (0)	-
BMI, ($\overline{X}$ ± SD)	20.65 ± 2.32	21.59 ± 2.56	19.55 ± 2.40	> 0.05 [d]
Dyslipidemia, n (%)	23 (85.2)	43 (89.6)	7 (87.5)	> 0.05 [e]
Hemoglobin (g/L), ($\overline{X}$ ± SD)	137.46 ± 15.46	134.66 ± 13.05	121.12 ± 13.10	< 0.05 [d]
Anemia, n (%)	6 (23.1)	14 (29.2)	6 (75)	< 0.05 [e]
Ure (mmol/L), ($\overline{X}$ ± SD)	6.12 ± 1.48	5.64 ± 1.57	5.69 ± 2.18	> 0.05 [d]
Creatinine (µmol/L), ($\overline{X}$ ± SD)	99.91 ± 19.91	103.58 ± 23.25	97.27 ± 29.56	> 0.05 [d]
eGFR (ml/phút), Median (Quartiles)	76 (67 - 92)	69 (64.25 - 82.50)	81 (65.25 - 94.50)	> 0.05 [c]
Uric acid (µmol/L), Median (Quartiles)	430 (346.9 - 473.6)	385.80 (338.22 - 459.05)	289.95 (271.97 -364.27)	< 0.05 [c]
Cholesterol (mmol/L), ($\overline{X}$ ± SD)	4.84 ± 1.16	4.83 ± 1.23	4.31 ± 0.71	> 0.05 [d]
LDL-C (mmol/L), ($\overline{X}$ ± SD)	3.12 ± 0.84	3.23 ± 0.97	2.74 ± 0.44	> 0.05 [d]
AIP, Median (Quartiles)	0.18 (-0.45 - 0.44)	0.30 (0.14 - 0.57)	0.16 (0.09 - 0.48)	> 0.05 [c]
Hs-CRP (mg/L), Median (Quartiles)	0.57 (0.32 - 0.94)	0.95 (0.47 - 2.47)	0,46 (0.30 - 0.69)	< 0.05 [c]

[c] Kruskal-Wallis test; [d] One-way ANOVA test; [e] Chi-square test

At 6 months after transplant, low hemoglobin levels, high rate of anemia, low blood uric acid, and hs-CRP levels were identified as independent determinants of plasma ADMA raised from normal to high levels.

Table 4: Logistic multivariable analysis of pre-transplant hemoglobin, ure, and hs-CRP associated with increased ADMA levels after Tx.

Indexes	Odds ratio (OR)	Confidence interval 95%	p
Hemoglobin (g/L)	0.964	0.933 - 0.996	< 0.05
Ure (mmol/L)	1.077	1.005 - 1.154	
Hs-CRP (mg/L)	1.358	1.067 - 1.728	

Among pre-transplant determinants, only hemoglobin, urea, and hs-CRP levels were independent determinants associated with increased ADMA levels at 6 months after Tx, $p < 0.05$. Previous studies have shown that increases in ADMA levels were consistent with an increase in cardiovascular events and graft rejection prediction. Multivariate analysis of pre-transplant determinants affecting plasma ADMA variation showed a correlation between the increase of ADMA levels and increase of CRP, decrease in hemoglobin, and increase in pre-transplant urea levels. Hs-CRP was a specific inflammatory marker, promoting atherosclerosis formation, increasing oxidative stress, and accumulating end-products, including ADMA, in agreement with Pihlstrom et al. [2].

Table 5: Clinical and laboratory factors related to increased ADMA levels after Tx.

Determinants	Odd ratio (OR)	Confidence interval 95%	p
Hypertension	5.01	1.17 - 21.44	< 0.05
Hemoglobin	0.94	0.83 - 0.99	< 0.05
Uric acid	0.99	0.99 - 1.00	> 0.05
Cholesterol	0.19	0.04 - 0.87	< 0.05
Triglycerid	5.70	1.20 - 16.26	< 0.005
LDL-C	7.85	1.04 - 59.02	< 0.05
Hs-CRP	1.45	0.10 - 2.11	> 0.05

In multivariate analysis, we found hypertension, hemoglobin, cholesterol, triglycerides, and LDL-C levels were independent determinants associated with an increase in ADMA levels at 6 months after Tx, $p < 0.05$. However, multivariate analysis data also indicated a positive correlation between hypertension, an increase in cholesterol, triglycerides, LDL, and a negative correlation between hemoglobin and an increase in ADMA levels, $p < 0.05$. Previous studies were consistent with our data, including Sadollah Abedini [6] and Pihlstrom [2]. These data suggest that despite controlled blood pressure following Tx, but indicate that even increases of ADMA levels are associated with worse outcomes. In patients with CKD, the reduction/loss of kidney function causes accumulation of lipid components and promotes the formation of atherosclerosis, leading to disturbances in lipid metabolism. However, after Tx, dyslipidemia presents partly due to the use of immunosuppressive agents, diet, and weight gain. Therefore, it is very important to control weight after Tx, and gentle exercise will avoid weight gain, reduce obesity, insulin resistance, the risk of lipid disorders, and cardiovascular events [7]. Regarding hemoglobin levels, our findings indicate that a decrease in hemoglobin levels after Tx will elevate plasma ADMA. Red blood cells have an important function to store ADMA, so the lysis of red blood cells releases a large amount of free ADMA, thereby promoting the degradation of methylated proteins [8]. Anemia in CKD patients is caused by decreased production of erythropoietin by the kidneys, nutritional deficiencies, bleeding during filtration, inflammation, and metabolic disorders. After successful renal Tx, a transient rise in the immediate post-operative period followed by a subtle decline, hemoglobin returned to normal. However, there were still some cases of anemia due to bleeding during surgery and viral infection.

CONCLUSION

Study on evolution of plasma ADMA levels in 83 patients at 6 months after Tx compared with time point before Tx and 83 healthy people of similar age and gender, we draw some following conclusion:

- Plasma ADMA levels at 6 months after Tx was 0.49 µmol/L showed a steep decline than before Tx (0.68 µmol/L), but was also higher than levels observed in the control group (0.32 µmol/L), $p < 0.001$.

- The median ADMA change was 0.17 µmol/L, of which 32.5% of recipients returned to normal and 9.6% of recipients revealed the transient increase of ADMA levels after 6 months Tx.

- Hemoglobin and hs-CRP levels before Tx; hypertension and dyslipidemia (especially elevated triglyceride) after Tx are independent determinants associated with increased plasma ADMA after 6 months Tx.

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