Original Article

Predictive role of methylarginines in renal failure

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Abstract

Aim: In this study, we aimed to investigate the role of methylarginines in renal failure and the process leading to failure. Acute Renal Failure (ARF) is defined as sudden onset, damage and loss of renal function. Chronic Kidney Disease (CKD) is generally defined as the presence of decreased renal function loss that persists for more than 3 months. Methylarginines are arginine derivatives which inhibit ischemia and vasoconstriction by inhibiting nitric oxide synthesis, causing ischemia and damage. Methylarginines are metabolized mainly in the kidney. Their metabolism is in the form of direct excretion and enzymatic degradation.

Material and Methods: The study was conducted from 2017 through 2018 in 89 patient (serum creatinine levels above 1.5 mg/dL) and 54 healthy control cases. Methylarginines were analyzed in positive mode from Turbo Ion Spray Electrospray (ESI) using ABSCIEX API 3200 High Performance Liquid Chromatography mass spectrometry (LC-MS / MS) instrument using C18 column. Urea and creatinine were analyzed by Beckman Coulter AU5800 Series. Results were evaluated with SPSS system.

Results: A significant difference was found in terms methylarginines urea and creatinine when patient and control groups compared. However, only creatinine and creatinine clearance values differences were found in patient groups in terms of acute and chronic renal failure (respectively, p = 0.001 for creatinine and p = 0.028 for creatinine clearance).There was no difference in terms of other parameters.

Conclusions: Methylarginines can be considered as new and high potential biomarkers for evaluating renal function. However, it is not considered to be adequate for show the prognosis of the disease.

INTRODUCTION

Acute renal failure (ARF) is defined as sudden loss of function in renal functions. In ARF, the diagnosis is made with the increase of the serum creatinine more than 0.3 mg/dL within 48 hours, more than the 50% increase of serum creatinine level compared to the previous week and the volume of urine output below 0.5 mL per kilogram hour during 6 hours. It is adequate to have any of these three findings for diagnosis (1).

Chronic kidney disease (CKD) is generally defined as the presence of ongoing renal damage and decreased renal function loss for more than 3 months (2).

CKD is characterized by progressive parenchymal damage and permanent nephron loss. Cardiovascular morbidity and mortality are significantly increased in patients with CKD. However, the mechanism of increased cardiovascular risk in CKD is unclear (2). Functional nephron loss stimulates growth of residual nephrons and increase of functional nephron capacity. The prolongation of these mechanisms triggers permanent damage. Permanent damage may cause separation of podocytes from the glomerular basement membrane and permanent loss of function. This functional loss is linked to interstitial fibrosis in the kidney. Early diagnosis of CKD is important in the progression of chronic renal failure, prevention or slowing of the course of cardiovascular diseases (3). If CKD can be diagnosed earlier, its progress can be modified, and its complications can be reduced (4).

Asymmetric dimethylarginine (ADMA), Symmetric dimethylarginine (SDMA) and N-monomethyl L-arginine (L-NMMA) are endogenous nitric oxide synthase inhibitors. ADMA, SDMA and L-NMMA are recognized as risk factors for various cardiovascular diseases such as hypertension, coronary artery disease, atherosclerosis, pulmonary hypertension, atrial fibrillation, stroke, peripheral vascular diseases, diabetes and congestive heart failure. The increase of these markers has been associated with an increase in cardiovascular deaths with C-reactive protein (CRP) and brain natriuretic peptide (BNP) (5).

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ADMA is a naturally occurring L-arginine analogue that can be used as a biomarker in kidney disease (6,7). ADMA inhibits nitric oxide (NO) synthesis endogenously and induces atherosclerosis by triggering endothelial dysfunction (7,8). Higher ADMA levels prevent endothelial nitrite formation. In kidney diseases and hypertension, as a result of decrease of arterial endothelial nitric oxide synthase (eNOS) phosphorylation deterioration in insulation endothelial functions occurs (6). SDMA is a stable degradation product of posttranslational proteins containing methylated arginine. SDMA is mainly metabolized by the kidneys (9).

There are many studies showing the correlation between renal dysfunction and increase in SDMA levels (10). Studies have shown that muscle mass, diet, inflammation, diabetes levels do not have a significant effect on SDMA concentration. In addition, SDMA levels have been shown to be unaffected by acute inflammation liver disease, cardiovascular disease, or diabetes if there is no concurrent kidney disease (11).

It has been shown in cell culture studies that inhibition of proteasome activation decreases the free forms of ADMA and SDMA. However, only ADMA decreased with inhibition of autophagy (12). Although SDMA cannot directly inhibit NO synthase, it may indirectly reduce NO production by causing intracellular arginine deficiency. However, SDMA reduces the transfer of arginine from the endothelial cell membrane into the cell (13).

SDMA acts by indirectly inhibiting NO release by increasing renovascular pressure and reducing renal blood flow (14). Studies reveal the increase in ADMA and SDMA in CKD. This level is higher in patients with end-stage of renal failure (15). Recently, disruption of ADMA and SDMA metabolism has been shown to play a role in the etiopathogenesis of cardiovascular diseases and chronic kidney disease. ADMA and L-NMMA, the family of dimethylarginine dimethylaminohydrolose-1 (DDAH1), is excreted from the body by destruction or through renal excretion(16).

ADMA and L-NMMA can also increase free oxygen radicals such as O_2^- and $ONOO^-$. L-arginine deficiency can also disrupter NOS (17).

In this study, we investigated the relationship between renal function and arginine and arginine metabolites in patients with renal insufficiency, healthy subjects, patients with ARF and patients with CKD. We aimed to investigate the role of arginine metabolites in assessing renal function and determining the course of renal function loss and to predict whether the levels of the arginine metabolites and the kidney damage are permanent or not.

MATERIAL and METHODS

The non-dialysis patients who were admitted to the nephrology clinic of Selcuk University Faculty of Medicine in 2017 and 2018 with creatinine values higher than 1.5 mg / dL were included in the study. Patients were grouped according to renal failure as ARF and CKD. As a control group, individuals who had general health examination in our and no disease were included in our hospital. Blood samples were collected from the patient and control groups from the antecubital region in the sitting position from the brachial vein to vacutainer and gel-filled tubes and EDTA tubes. Samples were taken in the morning after 12 hours of fasting. Routine tests were performed in the biochemistry laboratory. Serum samples taken from the blood samples for routine tests were portioned into Eppendorf tubes in our hospital's biochemistry laboratory and stored at -80 °C until the study day. On the study day, the samples were thawed at -20 °C, + 4 °C and at room temperature for appropriate periods.

For analysis of ADMA, SDMA, LNMMA, arginine and citrulline, the prepared plasma was studied with Turbo Ion Spray Electrospray (ESI) on positive mode at ABSCIEX API 3200 High Performance Liquid Chromatography mass spectrometry (LC-MS/MS) device by using Phenomenex Luna 50x4.6,5μ C18 HPLC column in biochemistry laboratory of our hospital. Two mobile phases were used during the analysis. A gradient is formed with the high-performance liquid chromatography containing formic acid (HPLC) of 0.1% grade water at the pump A and methanol flow containing 0.1 % formic acid at the pump B. Gradient is made according to the pump B. During the study, d7 isotope (Cambridge isotope DLM-7476-5) was used to prevent analyte losses during pre-analysis procedures. An internal standard was added to the standards prepared at different concentrations and a calibration graph was created. Standards were prepared based on mg / dL. Calibration graph was prepared with the results of analysis. Patient samples were analyzed according to the calibration graph.

Data were analyzed using SPSS statistical package program version 21 for Windows (SPSS Inc., Chicago, IL, USA). Variables were presented as mean ± standard deviation (SD) or frequency. Kolmogorov-Smirnov test was applied to evaluate the distribution of normality of the data beforehand to ensure that the data were subjected to appropriate statistical tests. In addition to descriptive statistics (mean ± SD, frequency), Student's t test was used to compare quantitative variables and Mann-Whitney U test was also used if variables were not normally distributed. Significance value was accepted as $p < 0.05$.

RESULTS

The study included 44 patients with ARF, 45 patients with CKD and a total of 89 renal failure patients. Fifty- four healthy subjects were included as the control group. The patients included in the study are composed of 26 women and 63 men. In the control group there are 19 women, 35 men.

When the patient and control groups were compared, significant differences were found between the groups

in terms of creatinine clearance, ADMA, SDMA, LNMMA, citrulline, creatinine and urea. The parameters with significant differences except creatinine clearance were higher in the group with renal failure compared to the control group. Creatinine clearance were lower in the renal failure group than the control group.

When the group with RF was grouped as ARF and CKD, a significant difference was found only in serum creatinine level and creatinine clearance/24h. There was no difference in other parameters. Serum creatinine level was higher in CKD group than ARF group.

Table 1. Comparison of renal failure patients and control groups

Parameters	Patient Group (n=89) Mean± SD	Control Group (n=54) Mean± SD	p-Value
Age	67.41±8.69	64.56±8.14	0.080
Creatinine clearance /24h	46.85±25.76	104.24±22.07	0.000*
ADMA (µmol/L)	0.39±0.16	0.23±0.07	0.000*
SDMA (µmol/L)	0.38±0.21	0.15±0.06	0.000*
LNMMA (µmol/L)	0.23±0.02	0.03±0.01	0.004*
L-Arginine (µmol/L)	95.36±49.29	100.34±47,77	0.577
Citrulline (µmol/L)	29.50±20.71	22.54±13.42	0.023*
Creatinine (mg/dL)	2.24±1.05	0.78±0.12	0.000*
Urea (mg/dL)	99.64±45.88	31.35±7.05	0.000*
* p <0.05			

Table 2. Comparison of ARF patients and CKD patients

Parameters	ARF Group (n=44) Mean±SD	CKD Group (n=45) Mean±SD	p-Value
Age	67.41±8.68	68.72±9.18	0.501
Creatinine clearance /24h	64.3±31.2	39.2±0.14	0.028*
ADMA (µmol/L)	0.37±0.17	0.34±0.07	0.194
SDMA (µmol/L)	0.37±0.23	0.4±0.17	0.144
LNMMA (µmol/L)	0.328±0.19	0.326±0.12	0.160
L-Arginine (µmol/L)	87.3±49.3	89.9±44,5	0.548
Citrulline (µmol/L)	24.19±18.3	33.5±19.8	0.862
Creatinine (mg/dL)	1.9±0.57	2.5±1.25	0.001*
Urea (mg/dL)	67.18±8.68	68.92±9.17	0.654
* p <0.05			

DISCUSSION

In this study, we investigated creatinine clearance, ADMA, SDMA, L-NMMA, L-arginine, citrulline, creatinine and urea in healthy control and renal failure groups in the same age range. Patient and control groups were compared for the analyzed tests examined. As a result of this comparison, a significant difference was found in other parameters except L-arginine. Other tests except creatinine clearance were higher in the patient group. Creatinine clearance was found to be lower in patients due to renal failure. When the patients with renal failure were grouped as acute and chronic, no significant difference was found in other parameters except serum creatinine and creatinine clearance level. Serum creatinine level was higher in CKD patients. Also, serum creatinine clearance level was found to be low in CKD patients.

Although it has been expressed that there is a relationship between methylarginine levels and vascular damage in patients with chronic renal failure, there is no definitive clinical data showing the relationship with death due to cardiovascular disease and renal failure as a result of methylarginine derivatives. However, the increase in methyl arginine is considered as an important risk factor for death (18). It has been shown in the studies that metabolites such as ADMA and SDMA are risk factors for cardiac and renal diseases. Insa E. Emrich et al. found a significant positive correlation between ADMA, SDMA and LNMMA levels and urea and albuminuria levels. There are studies suggesting that high ADMA levels can predict the course of chronic renal failure (8).

There are also studies showing that SDMA is more important than ADMA in the evaluation of renal failure. However, SDMA has been found to be inadequate in predicting the course of the disease in mild and moderate chronic renal disease patients who are stages of end-stage renal failure since SDMA is metabolized by excretion from the kidneys (19).

Kidneys, first by providing direct excretion of ADMA and secondly by providing enzymatic degradation of ADMA, play two types of role in ADMA metabolism. The slow ADMA decline and increase after hemodialysis in renal failure patients is due to insufficiency of enzymatic degradation and lack of urinary excretion (20).

Said MY et al., in their study, they found serum creatinine, serum urea levels and serum ADMA levels correlated as similar to our results. They also revealed that serum ADMA levels were correlated inversely with creatinine filtration rate. Although excretion of SDMA from methylarginines is more related to urinary mortality, there is a strong correlation between plasma methylarginine levels and plasma creatinine levels. There is also a relationship between urea excretion and plasma ADMA levels and mortality rates (21). In their study, Maas R. et al. found that the allopurinol use decreased plasma ADMA levels to a minimum, but had no effect on SDMA. They attributed this to the permanent damage of glomerular filtration in CKD and the variation of damage along the enzymatic metabolism pathway (22).

ADMA causes ischemia and oxidative stress by increasing polymorphonuclear cell activation, superoxide dismutase and myeloperoxidase release in patients with hyperuricemia. Like other methylarginines in SDMA, it can cause inflammation through leukocyte activation. There are even studies showing that the relationship between SDMA and inflammation is higher than ADMA (23,24). Clinical studies showed a correlation between the severity of the disease and methylarginine levels (24).

In our study, similar to the literature, methylarginines and urea and creatinine levels were found lower in the control group compared to the patient groups. However, there was no difference between the patient groups in methylarginines. We consider that this is due to the fact that renal excretion and enzymatic degradation of methylarginines occur in less than the time required to diagnose acute and / or chronic renal disease (renal injury for more than 3 months). Therefore, we think that these parameters are not sufficient to make an acute-chronic distinction of kidney diseases and to predict the onset of the disease. However, we think that levels of methylarginine are important parameters to show the severity of the disease or even the immediate effects of the disease on vital functions. Plasma levels of SDMA, one of the methylarginines, were found to be higher in CKD patients than in the ARF group, although not significantly. It was thought that SDMA elevation may be due to decrease of renal excretion in accordance with creatinine clearance. We think that elevated serum creatinine levels in patients with CKD are related to the level of kidney damage.

CONCLUSION

Methylarginines can be important diagnostic tests in identifying renal diseases. They show a strong correlation with serum creatinine level and inverse correlation with creatinine clearance. The relationship of these markers with creatinine suggests that methylarginines may be considered as diagnostic candidate markers in RF. However, due to the lack of difference between ARF and CKD, we conclude that methylarginines cannot be sufficient to predict recovery or end-stage renal failure in renal patients. However, the difference between the patient and control groups in assessing renal function has suggested that methylarginines are important alternative parameters in predicting survival of patients. In this study, the lack of long-term follow-up of patients and the absence of periodic measurement results in disease follow-up can be considered as limitations of the study. We believe that healthier results can be achieved by making long term and periodic measurements in larger patient groups.

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