






Evaluation of amino acid clearances in post-transplant kidney patients stratified by eGFR

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ABSTRACT: The human kidney has a prominent role in the metabolism of amino acids and the control of plasma concentrations. Treatment modalities, such as kidney transplantation, hemodialysis, etc., may alter amino acid concentrations. In the present study, we aimed to evaluate the effect of kidney transplantation on the amino acid profile and the relationship between creatinine clearance and amino acid clearances in post-transplant kidney patients. Serum and urine samples were obtained from 133 patients after kidney transplantation. Patients were divided into three groups based on eGFR. Amino acid profiles were performed by liquid chromatography coupled with tandem mass spectrometry, and clearances were calculated using the 24-hour creatinine clearance formula. The amino acid clearances between the studied groups differed significantly in 10 of the 27 amino acids. While 1-methyl-L-histidine, 3-methyl-L-histidine, Arginine, Cystine, Ethanolamine, Glycine, L-Histidine, Isoleucine, and Taurine significantly showed an upward trend, L-Proline especially revealed a downward trend. Amino acid clearances that showed the strongest correlation with creatinine clearance were 1-methyl-L-histidine, 3-methyl-L-histidine, and ethanolamine. However, Arginine and L-proline had a weak positive and negative linear relationship, respectively. This study showed that amino acid clearances varied in kidney transplant patients among the studied groups. Increased or decreased amino acid clearances are affected by the progression of renal dysfunction. These results give insight into kidney transplantation-associated modifications of amino acid metabolism.

KEYWORDS: Amino acids; kidney transplantation; amino acid clearance; estimated glomerular filtration rate; creatinine clearance.

1. INTRODUCTION

Globally, there are over 850 million individuals affected by kidney diseases, including chronic kidney disease (CKD), acute kidney injury (AKI), and those undergoing renal replacement therapy. These kidney conditions encompass a broad range of diseases with varying causes, clinical trajectories, and degrees of functional impairment. They span from cases of AKI to the different stages of CKD, ranging from stage 1 to stage 5, and may ultimately lead to end-stage kidney disease, necessitating chronic dialysis or kidney transplantation [1]. Between 1990 and 2017, there was a significant 41.5% increase in the global mortality rate associated with CKD [2]. Notably, CKD has risen through the ranks to become one of the top causes of death, progressing from the 36th position in 1990 to the 19th position in 2013, and further to the 12th position in 2017 [2–4]. Projections indicate that CKD is on track to become the fifth leading cause of years of life lost worldwide by 2040[4]. When CKD progresses to End Stage Kidney Disease, there are two primary treatment options: dialysis, a process for eliminating waste products and excess fluid from the blood, and kidney transplantation. Kidney transplantation is considered the preferred therapy for eligible patients with kidney failure due to its associated enhancements in both survival rates and quality of life.

The kidneys are responsible for maintaining the balance of amino acids in the bloodstream by filtering excess amino acids and reabsorbing those that are needed. The primary function of the kidney tubules

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concerning amino acids is to prevent their excretion in the urine [5]. Amino acids, essential for various bodily functions like protein synthesis, energy generation, and neurotransmitter production, are the fundamental components of proteins [6]. Approximately 99.5% of amino acids are reabsorbed, resulting in a fractional excretion of less than 0.5% of the filtered load [7]. In individuals with impaired kidney function, The clearance of amino acids from the bloodstream may be affected. This can lead to elevated levels of certain amino acids in the blood and disrupted pathways [8]. The aim of this study is to investigate the association between amino acid clearance and creatinine clearance in post-transplant patients, particularly among subdivided groups based on the estimated glomerular filtration rate (eGFR). To our knowledge, this relationship has not been previously explored in this patient population. Understanding the relationship between amino acid and creatinine clearance could provide valuable insights into renal function post-transplantation and potentially inform clinical management strategies for improving patient outcomes.

2. RESULTS

2.1. Patient baseline characteristics

Table 1 provides an overview of the clinical and demographic characteristics of the study participants. Our cross-sectional study included a cohort of 133 kidney transplant recipients, comprising 19 males (34.55%) with a mean age of 47.0 ± 13.2 years in Group 1, 16 males (29.09%) with a mean age of 46.6 ± 10.5 in Group 2, and 13 males (43.48%) with a mean age of 51.7 ± 14.0 in Group 3. The participants were stratified based on tertiles of GFR distribution, resulting in the following groups: eGFR >60 mL/min/1.73 m² (Group 1), $30 < \text{eGFR} < 60$ mL/min/1.73 m² (Group 2), and eGFR <30 mL/min/1.73 m² (Group 3). The mean/median values of BMI, cholesterol, triglycerides, and CRP among individuals in all groups fell within the normal range. Additionally, there were no significant variations in demographic characteristics, lipid profiles, BMI, CRP, prevalence of diabetes mellitus, hypertension, smoking history, or body surface area among the different groups (see Table 1).

2.2. Differential Amino Acid Analysis

The analysis of amino acid and creatinine clearance profiles using a non-parametric test revealed notable distinctions among groups in 10 out of the 27 amino acid clearances, as indicated in Table 2. Compared with those in groups, the clearance levels of 1-methyl-l-histidine ($P = 9.03\text{E-}15$), 3-methyl-l-histidine ($P = 3.98\text{E-}16$), arginine ($P = 0.049$), cystine ($P = 0.0004$), ethanolamine ($P = 3.98\text{E-}16$), glycine ($P = 0.019$), L-Histidine ($P = 0.00001$), Isoleucine ($P = 0.025$), Taurine ($P = 0.0006$), were significantly increased and showed a downward trend (from Group 1 to Group 3), L-Proline ($P = 0.006$), on the other hand, significantly revealed a downward trend. There was no significant difference in the levels of alanine, citrulline, glutamic acid, glutamine, hydroxyproline, leucine, lysine, methionine, ornithine, phenylalanine, serine, threonine, tryptophan, tyrosine, and valine amino acids between groups.

Table 1. Patient Demographics Following Kidney Transplantation. The data are presented as mean \pm SD, median (25th, 75th percentiles), or counts n and n (%). M represents Male, while F represents Female. The measured parameters include eGFR (Glomerular Filtration Rate), BMI (Body Mass Index), BUN (Blood Urea Nitrogen), and NS indicates not significant.

Patient characteristics	Group 1 (n=55)	Group 2 (n=55)	Group 3 (n=23)	p-value
Age (years)	47.0 \pm 13.2	46.6 \pm 10.5	51.7 \pm 14.0	NS
Gender (M/F)	36/19	39/16	10/13	NS
Smoking status (%)				NS
Never	63.6	63.6	78.3	

<i>Former</i>	18.2	9.1	8.7	
<i>Current</i>	18.2	27.3	13.0	
<i>BMI (kg/m2)</i>	25.46 (24.0-29.6)	26.7 (23.7-29.4)	27.6 (24.3-30.4)	NS
<i>eGFR(mL/min/1.73m2)</i>	76 (66-88)	47 (41-54)	18 (15-24)	3.38E-25
<i>BUN (mg/dL)</i>	24 (17.5-37)	22 (15-28)	23 (19-57)	
<i>Creatinine clearance (mg/dL)</i>	72.8 (57.7-94.5)	38.5(32.8-47.9)	18 (11.9-21.7)	9.62E-19
<i>CRP</i>	0.37 (0.15-0.80)	0.30 (0.13-0.79)	0.38 (0.14-0.91)	NS
<i>Cholesterol, LDL(mg/dL)</i>	104.5 (82.5-130.2)	111.0 (81.5-135.5)	111.0 (93.0-141.0)	NS
<i>Triglyceride (mg/dL)</i>	141.5 (91.0-230)	141 (106-181)	155 (115-283)	NS
<i>Diabetes Mellitus (%)</i>	7.4	9.1	39.1	NS
<i>Hypertension (%)</i>	9.3	7.3	18.2	NS

Table 2. Amino acid clearance between groups. The data are presented as median (25th, 75th percentiles), NS: not significant.

<i>Variables (umol/L)</i>	<i>Group 1 (n=55)</i>	<i>Group 2 (n=55)</i>	<i>Group 3 (n=23)</i>	<i>P-value</i>
<i>Creatinine Clearance</i>	72.8 (57.7-94.5)	38.5 (32.8-47.9)	18.0 (11.9-21.7)	9.62E-19
<i>1-Methyl-L-Histidine</i>	27.4 (17.1-49.7)	11.3 (8.54-16.7)	5.12 (3.53-8.44)	9.03E-15
<i>3-Methyl-L-Histidine</i>	26.7 (18.1-40.6)	13.4 (10.3-17.2)	5.99 (4.37-8.23)	3.99E-16
<i>Alanine</i>	0.58 (0.43-0.92)	0.49 (0.27-0.65)	0.47 (0.29-0.90)	NS
<i>Arginine</i>	0.19 (0.13-0.26)	0.18 (0.14-0.27)	0.12 (0.09-0.21)	4.90E-02
<i>Asparagine</i>	0.69 (0.26-1.84)	0.64 (0.39-1.41)	0.38 (0.23-1.90)	NS
<i>Citrulline</i>	0.14 (0.08-0.30)	0.17 (0.10-0.35)	0.22 (0.07-0.43)	NS
<i>Cystine</i>	11.8 (2.57-29.7)	6.02 (2.72-9.96)	1.45 (0.38-4.69)	3.45E-04
<i>Ethanolamine</i>	34.6 (21.3-42.2)	18.6 (13.5-23.2)	8.84 (6.02-11.7)	1.53E-12
<i>Glutamic acid</i>	0.43 (0.15-0.63)	0.26 (0.16-0.42)	0.25 (0.13-0.61)	NS
<i>Glutamine</i>	0.46 (0.25-0.79)	0.35 (0.25-0.61)	0.34 (0.19-0.60)	NS
<i>Glycine</i>	2.93 (1.17-4.92)	1.62 (0.85-3.23)	1.69 (1.08-2.82)	1.90E-02
<i>L-Histidine</i>	5.07 (3.28-8.90)	3.20 (2.07-5.07)	2.09 (1.33-2.82)	4.00E-06
<i>Hydroxyproline</i>	0.44 (0.21-1.10)	0.64 (0.17-1.86)	0.84 (0.30-1.91)	NS
<i>Isoleucine</i>	0.13 (0.06-0.26)	0.11 (0.05-0.21)	0.07 (0.03-0.13)	0.025
<i>Leucine</i>	0.09 (0.00-0.21)	0.08 (0.00-0.17)	0.10 (0.00-0.20)	NS
<i>Lysine</i>	0.28 (0.17-0.66)	0.33 (0.15-0.59)	0.21 (0.14-0.42)	NS

<i>Methionine</i>	0.13 (0.05-0.20)	0.17 (0.08-0.25)	0.07 (0.04-0.17)	NS
<i>Ornithine</i>	0.18 (0.11-0.23)	0.18 (0.11-0.29)	0.09 (0.06-0.29)	NS
<i>Phenylalanine</i>	0.45 (0.28-0.77)	0.42 (0.25-0.68)	0.54 (0.23-0.93)	NS
<i>L-Proline</i>	0.03 (0.02-0.06)	0.06 (0.02-0.11)	0.08 (0.03-0.56)	6.00E-03
<i>Serine</i>	0.85 (0.11-2.06)	0.77 (0.33-1.67)	0.58 (0.02-1.48)	NS
<i>Taurine</i>	4.28 (1.69-10.2)	2.33 (0.99-4.73)	1.61 (0.60-2.95)	1.00E-02
<i>Threonine</i>	0.90 (0.70-1.63)	1.06 (0.66-1.58)	1.37 (0.58-1.97)	NS
<i>Tryptophan</i>	0.57 (0.40-0.97)	0.46 (0.28-0.68)	0.50 (0.24-0.83)	NS
<i>Tyrosine</i>	0.43 (0.14-0.75)	0.34 (0.14-0.80)	0.33 (0.17-0.98)	NS
<i>Valine</i>	0.05 (0.02-0.10)	0.05 (0.03-0.08)	0.06 (0.01-0.12)	NS

2.3. Correlation Analysis of Different Metabolites and Kidney Function Indicators

Spearman correlation analysis was utilized to examine the associations between creatinine clearance and significant amino acid clearances, including 1-methyl-l-histidine, 3-methyl-l-histidine, arginine, cysteine, ethanolamine, glycine, L-Histidine, Isoleucine, L-Proline, and Taurine. The results, as presented in the table, revealed robust correlations between 1-methyl-l-histidine ($r = 0.742$, $P = 2.23E-24$), 3-methyl-l-histidine ($r = 0.790$, $P = 1.46E-29$), and Ethanolamine clearance ($r = 0.732$, $P = 1.37E-23$) with creatinine clearance, all highly statistically significant. Arginine displayed a weak positive correlation ($r = 0.210$, $P = 0.015$), while L-Proline exhibited a weak negative correlation ($r = -0.224$, $P = 0.010$) with creatinine clearance. Furthermore, Glycine ($r = 0.323$, $P = 0.00015$), L-Histidine ($r = 0.469$, $P = 1.26E-8$), Cystine ($r = 0.435$, $P = 1.72E-7$), and Taurine ($r = 0.391$, $P = 0.000003$) demonstrated moderate positive linear relationships with creatinine clearance. In contrast, Isoleucine did not display a significant correlation with creatinine clearance ($r = 0.158$, $P = 0.069$).

2.4. Analysis of Metabolic Pathways

To explore potential metabolic pathways associated with post-transplant renal dysfunction, we harnessed the power of the MetaboAnalyst 5.0 program's Pathway Analysis tool. This software draws from databases like KEGG (<http://www.genome.jp/kegg/>), PubChem (<https://pubchem.ncbi.nlm.nih.gov/>), and the Human Metabolome Database (<http://www.hmdb.ca>) to generate correlation graphs that reveal affected metabolic pathways, accompanied by corresponding P-values. The program calculates a pathway's impact value by conducting a topology analysis that considers the significance of individual metabolites within that pathway. Metabolites that play pivotal roles within a pathway have a more substantial impact when their concentrations undergo changes. Furthermore, the program evaluates pathway significance through an enrichment analysis, assigning weights to each metabolite within a given dataset. Higher significance levels and elevated P-values indicate a more pronounced influence on metabolic flow. Consequently, the correlation graphs portray the significance in terms of impact on metabolic pathways vs. $-\log(p)$, with the size and color of circles denoting different levels of significance. The color gradient, transitioning from white to yellow and then to red, signifies increasing impact, as observed in the various graphs in Figure 1 [9].

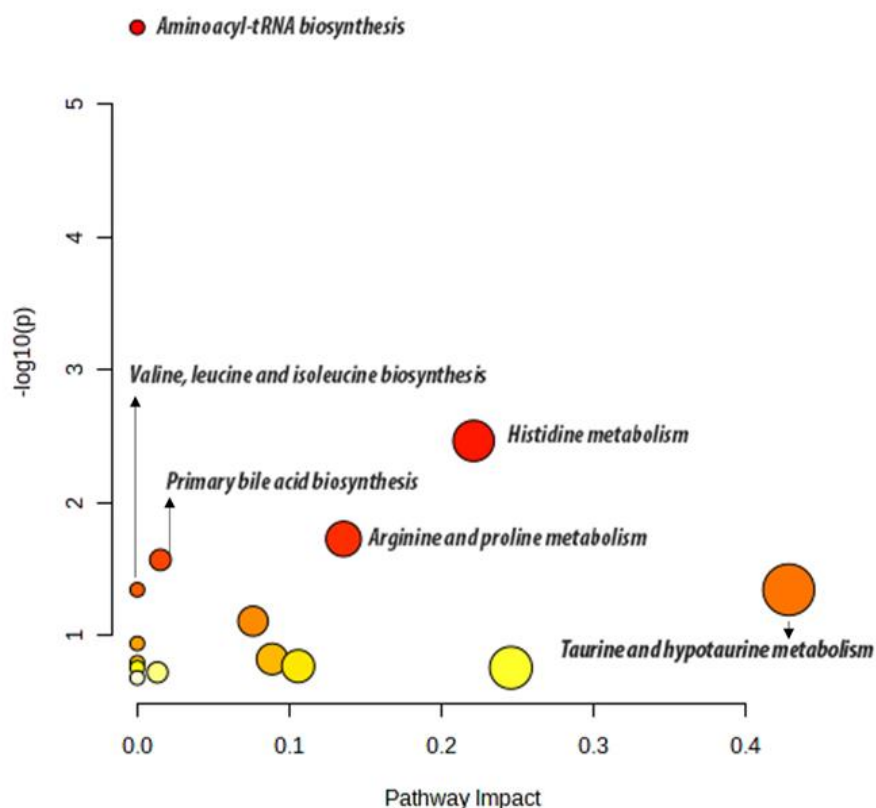


Figure 1. A graph illustrating the significance of metabolic pathway impact (pathway impact vs. $-\log(p)$) created utilizing the Pathway Analysis module within the MetaboAnalyst 5.0 program.

The top five metabolic pathways were selected based on their $-\log(P)$ value and pathway impact score: aminoacyl-tRNA biosynthesis, histidine metabolism, arginine and proline metabolism, primary bile acid biosynthesis, Valine, leucine, and isoleucine biosynthesis, and taurine and hypotaurine metabolism (Figure 1).

3. DISCUSSION

Amino acid clearance is intricately linked to the health of the kidneys. In individuals with well-functioning kidneys, amino acids are efficiently reabsorbed by the renal tubules, resulting in minimal amino acid excretion in the urine [7]. However, when kidney function is compromised, as in the case of CKD, clearance rates may be affected [8]. Following kidney transplantation, monitoring amino acid clearance becomes a valuable tool for assessing the performance of the transplanted kidney. A properly functioning transplanted kidney should regulate amino acid levels in the body in a manner akin to a healthy kidney. To the best of our knowledge, this is the first study that compared the creatinine clearance and amino acid clearances of post-transplant patients between groups subdivided based on eGFR. This study demonstrates the correlation between creatinine clearance and amino acid clearances in post-transplant outpatients.

Histidine is an essential amino acid with diverse functions, including hormone production, neurotransmission, and immune response regulation [10]. In CKD, histidine levels may decrease, leading to disruptions in histidine metabolism and related byproducts like histamine. CKD patients often exhibit altered urinary amino acid concentrations. Research indicates that low plasma histidine levels are associated with oxidative stress, protein-energy wasting, and inflammation [11]. Histidine plays crucial roles in enzymatic functions and can be subject to enzymatic methylation. The excretion of 3-methylhistidine in urine, stemming from contractile proteins, serves as an indicator of muscle degradation [10]. In CKD, elevated plasma 3-methylhistidine levels suggest increased muscle breakdown and impaired excretion. Similarly, 1-methylhistidine, predominantly located in skeletal muscle, is released into the bloodstream during muscle protein breakdown. This amino acid serves as an indicator of muscle protein catabolism. In

the context of CKD, it holds relevance as CKD patients often experience muscle wasting or sarcopenia. This muscle loss can result from factors such as inflammation, malnutrition, and reduced physical activity due to illness, contributing to the release of amino acids like 1-methylhistidine into the bloodstream. A study showed that CKD leads to reduced histamine, 3-methylhistidine, and L-histidine in urine, indicating disruptions in histidine metabolism [10]. At the conclusion of our study, we noted a reduction in histidine, 1-methylhistidine, and 3-methylhistidine clearance levels in groups with lower eGFR. Strong positive correlations exist between 1-methylhistidine and 3-methylhistidine clearance and creatinine clearance, implying reduced clearance as kidney function declines. A moderate positive correlation between histidine clearance and creatinine clearance suggests a moderate influence of kidney function on histidine clearance.

Taurine, a vital amino sulfonic acid abundant in cells, serves various physiological roles including osmoregulation, membrane stability, and neurotransmission [12,13]. In human, it plays a part in kidney diseases like glomerulonephritis, diabetic nephropathy, renal failure, and acute kidney injury, often studied in animal models [14]. Taurine exhibits anti-inflammatory properties and shields cells from inflammation-induced damage [15]. Notably, decreased taurine clearance was observed in the low eGFR group in our study. The observation of significantly decreased taurine clearance in the low eGFR group suggests a potential link between reduced kidney function (as indicated by low eGFR) and impaired taurine handling by the kidneys. This finding aligns with previous research indicating that taurine plays a role in kidney function and protection. In the context of kidney disease, reduced taurine clearance could imply that the kidneys are less efficient at excreting taurine. This could be due to compromised kidney function, as seen in chronic kidney disease and acute kidney injury. Taurine is known to have protective effects on kidney cells and to be involved in anti-inflammatory processes, so its altered clearance may be related to the kidney's response to injury or dysfunction. Further research is needed to fully understand the implications of decreased taurine clearance in individuals with low eGFR. It's essential to consider this finding in the context of other clinical and biochemical parameters to determine its significance for kidney health and disease progression.

L-Arginine is a semi-essential amino acid synthesized from glutamine, glutamate, and proline through the intestinal-renal axis in humans and many mammals. The breakdown of L-Arginine follows multiple pathways, generating essential biological molecules such as nitric oxide, polyamines, proline, glutamate, creatine, and agmatine. Imbalances in L-arginine and its metabolites are linked to several diseases, including kidney and cardiovascular conditions, where these metabolites might contribute to the disease's development. It is documented that L-Proline, metabolized in vivo by L-arginine, is a substrate for collagen synthesis and is involved in tissue repair with L-arginine and other metabolites. In addition, L-Proline and metabolites in the pathway can reduce kidney damage by reducing oxidative stress [16]. The our study's specific findings indicate that there was a significant upward trend in L-Proline levels when comparing Group 1 with Groups 2 and 3. At the same time, the level of arginine showed a downward trend. This suggests that in individuals with different levels of kidney function (as indicated by eGFR), there are alterations in the levels of these amino acids. Such alterations might have implications for tissue repair, oxidative stress, and potentially the development or management of kidney and cardiovascular diseases.

Ethanolamine, which is derived from the non-essential amino acid serine, plays a role as the head group of phosphatidylethanolamine in cell membranes [17]. CKD is associated with oxidative stress, mitochondrial dysfunction, and chronic inflammation. Ethanolamine has been of interest in CKD research due to its potential anti-inflammatory and antioxidant properties [18]. Lowering chronic inflammation and inhibiting oxidative stress, which are closely linked to NF- κ B activation, are important in managing CKD and its related disorders [19]. Ethanolamine might offer benefits to CKD patients, potentially at both early and late stages of the disease. Glycine, on the other hand, is one of the amino acids, and its role in CKD relates to its presence in urine. Research has suggested that low levels of glycine in urine could indicate a risk of AKI. In studies, both glycine and ethanolamine showed down-regulation in patients with. [17]. Consistent with this study, we experienced a decrease in urinary glycine and ethanolamine levels and a decrease in their clearance as eGFR decreased. In summary, ethanolamine and glycine are metabolites that have shown associations with kidney health, particularly in the context of CKD and AKI. Further research is needed to fully understand their roles in kidney diseases.

Cysteine, an amino acid crucial for various bodily functions, can be acquired through diet or synthesized from methionine [20]. It primarily exists in its oxidized forms, including cystine. Kidney dysfunction or reduced cysteine clearance can elevate circulating cystine levels. Patients on hemodialysis often exhibit increased cystine levels due to impaired kidney function, impacting redox control and clearance functions [21]. In our study, we observed decreased cystine clearance as eGFR declined among

different groups. The limitations of this study include a small sample size and a single-center design. Nevertheless, despite these constraints, our preliminary investigation can provide valuable insights and serve as a guide for future research endeavors.

4. CONCLUSION

The amino acid clearance profiles, calculated based on serum and urinary amino acid profiles, were found to be altered in patients with kidney transplantation between the studied groups, specifically involving pathways such as aminoacyl-tRNA biosynthesis, histidine metabolism, arginine and proline metabolism, primary bile acid biosynthesis, and Valine, leucine, and isoleucine biosynthesis, as well as taurine and hypotaurine metabolism. The observed elevations or reductions in amino acid clearance levels, which correlated with creatinine clearance, were influenced by the progression of renal dysfunction and are indicative of renal function. These findings offer valuable insights into the modifications of amino acid metabolism associated with kidney transplantation, shedding light on potential therapeutic targets and strategies for managing post-transplant complications.

5. MATERIALS AND METHODS

5.1. Study Design and Population

In this study, we enrolled 133 renal transplant patients who met specific criteria: adults (age ≥ 18 years) with either living or cadaver kidney transplants, a willingness to comply with study procedures, and the provision of serum and 24-hour urine samples. Exclusions were made for patients with a history of infection, pregnancy, cancer, acute cardiovascular events, or blood diseases. Urine and blood samples were gathered from outpatients under the care of the Kidney Transplant Center at Bursa Acibadem Hospital. The post-transplant-eGFR was determined using the CKD-EPI 2021 formula based on serum creatinine levels. The patient cohort was divided into three groups: 55 individuals with eGFR >60 mL/min/1.73 m² (Group 1), 55 with eGFR ranging from 30 to 60 mL/min/1.73 m² (Group 2), and 23 with eGFR <30 mL/min/1.73 m² (Group 3). Amino acid clearances were calculated using the 24-hour creatinine clearance formula: CrC (in milliliters/minute per 1.73 m²) = [(urine volume \times urine Cr)/(serum Cr \times 1,440)] \times (1.73 m²/body surface) (9). Ethical approval for the study was obtained from the Acibadem Mehmet Ali Aydinlar University Human Scientific and Ethical Review Committee (Approval ID: 2020-08/14), and written informed consent was obtained from all study participants.

5.2. Demographic Data and Laboratory Measurements

Table 1 provides demographic details, including gender, age, BMI, smoking history, diabetes mellitus presence, and hypertension status. The hospital's laboratory conducted analyses on plasma and urine samples collected from all patients. Blood samples, obtained following an overnight fast, were promptly analyzed on the same day. The table also presents laboratory results such as C-reactive protein (CRP), direct LDL cholesterol, triglyceride levels, creatinine clearance, and eGFR calculated via CKD-EPI-2021. Biochemical and hematological parameters were evaluated using the Siemens Healthineers Dimension EXL 200 Integrated Chemistry System and the Sysmex XT-1800i automated analyzer. The manufacturer supplied all calibrators, reagents, and controls.

5.3. Amino acid analysis and LC-MS/MS conditions

5.3.1. Sample preparation

50 μ L of serum sample was treated with 50 μ L IS mix solution in Eppendorf tubes. The mixture was vortexed for 1 min. Then 1000 μ L mobile A and Mobile B (1:5) were added and mixed. After the centrifugation process supernatant was transferred to a vial, these steps were repeated for urine samples, but samples were diluted ten times before the initial step.

5.3.2. LC-MS Condition

For amino acid analysis in serum and urine samples, we employed an Agilent 6460 triple-quadrupole mass spectrometer, which was equipped with an electrospray ionization (ESI) source (Agilent Technologies,

Wilmington, DE, USA). Separation of compounds was carried out using a Zorbax SB-C18 column (150 mm × 3.0 mm, 3.5 µm, Agilent Technologies, USA) at a temperature of 30°C and a flow rate of 0.7 mL/min. The mobile phase A consisted of formic acid and ammonium formate, while mobile phase B was composed of acetonitrile. An injection volume of 3 µl was utilized in conjunction with a gradient elution system. Internal standards for amino acids were procured from Cambridge Isotope. Data was collected and assessed using multiple reaction monitoring techniques for amino acids.

5.3.3. Statistical Analysis

We conducted data analysis using the Statistical Package for the Social Sciences Software (SPSS for MacBook software version 21.0, IBM Corp., Armonk, NY, USA). All statistical tests were two-tailed, and we considered values of $P < 0.05$ as statistically significant. For normally distributed data, we present results as mean ± standard deviation, while non-normally distributed data is presented as median values with an interquartile range. Categorical variables are expressed as percentages. To compare clinical and demographic variables among groups, we employed the Kruskal-Wallis test, a non-parametric (distribution-free) test, for continuous variables, and the Chi-square test for categorical variables. Additionally, we utilized Spearman's rank analysis to examine the relationship between creatinine clearance and significant amino acids.

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Conflict of interest statement: The authors declare that they have no competing interests.

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