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ORIGINAL RESEARCH

Indole Lactic Acid in Plasma and Urine: A Potential Biomarker for Chronic Kidney Disease and Inflammatory

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Purpose: We aimed to explore changes in plasma and urine indole lactic acid (ILA) levels and the relationship between inflammation and ILA in chronic kidney disease (CKD) patients and healthy people.

Patients and Methods: Forty-seven CKD patients and 30 healthy individuals were included in this study. One-way ANOVA was used for variables with normal distribution and homogeneous variance. A rank-sum test was performed for non-normally distributed variables. Correlation analyses were performed using Pearson's or Spearman correlation analyses. Independent relationship between patients and CKD was analyzed using ordinal and binary logistic regressions. Receiver operating characteristic (ROC) curve was used.

Results: Plasma and urine ILA levels were positively correlated (r = 0.51, $P < 0.01$). Plasma ILA was positively correlated with BMI, age, creatinine, BUN, triglycerides, and uric acid and negatively correlated with hemoglobin levels. Urine ILA levels were positively correlated with age, creatinine, BUN, and uric acid and negatively correlated with hemoglobin and albumin levels. Ordered logistic regression analysis showed that CKD was significantly correlated with plasma ILA (OR=4.49, $P < 0.01$), urinary ILA (OR=2.14, $P < 0.01$), urea levels (OR=1.43, $P < 0.01$) and hemoglobin levels (OR=0.95, $P < 0.01$) were significantly related. ROC curves indicated that plasma and urinary ILA were reliable predictors of CKD. CKD was correlated with plasma, urine ILA (OR=5.92, $P < 0.01$; OR=2.79, $P < 0.01$) and Hs-CRP (OR=2.45, $P < 0.01$).

Conclusion: Plasma and urine ILA can potentially be used as biomarkers of CKD and inflammatory status.

Keywords: predictor, metabolomics, tryptophan metabolism, inflammation

Introduction

Chronic kidney disease (CKD) threatens the world, and a patient's quality of life can deteriorate with disease progression.[1] CKD pathogenesis involves inflammation, immune disorders, and oxidative stress.[2,3] The specific pathogenesis of CKD has not yet been fully elucidated. Various causes of CKD can involve different cells and sites of inflammatory damage. For instance, hypertension can lead to the leakage of mitochondrial DNA in renal epithelial cells and enhance the expression of stimulator of interferon genes, thereby regulating inflammation and the progression of CKD. IgA inflammation is caused by the formation of galactose-deficient IgA1 immune complexes in the glomerular mesangium.[4,5] In patients with Autosomal-Dominant Polycystic Kidney Disease, macrophage migration inhibitory factors can act as persistent inflammatory stimulators, influencing the progression of cysts.[6,7] Clarifying the pathogenesis of CKD is of great significance for disease development and prognosis.

Indole lactic acid (ILA) is a protein-bound uric acid solute derived from tryptophan metabolism and belongs to the indole uremic solute family.[8] ILA activates the aryl hydrocarbon receptor (AhR) transcription factor, which promotes inflammation, immune responses, and oxidative stress. It also plays a significant role in cardiovascular diseases. ILA has

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the potential to regulate intestinal homeostasis by reducing the accumulation of inflammatory macrophages.[9] By increasing H3K27ac binding at the enhancer regions of IL12a, ILA facilitated the augmentation of IL12a production in dendritic cells, thereby promoting the stimulation of CD8 T cell immunity against tumor growth. Additionally, ILA exhibited the ability to enhance the activity of CD8 T cells present within the tumor microenvironment by modulating chromatin accessibility.[10,11] Urinary toxin accumulation usually affects disease progression and prognosis in patients with CKD.[12] The presence of urine toxins in patients with CKD is associated with various complications and may contribute to cardiovascular events. This has raised concerns about the development of toxins in patients with CKD.[13]

Metabolomic methods are commonly used in clinical practice to detect urinary toxins.[14,15] This study aimed to explore the changes in plasma and urine ILA in patients with CKD and controls through LC-MS and the relationship between inflammation and ILA in patients with CKD and controls.

Materials and Methods

Participant selection of 47 CKD patients and 30 healthy individuals. Our inclusion criteria for screening patients were as follows: 1. The patient met the criteria for CKD diagnosis. 2. The patients were over 18 years old. We excluded pregnant women, lactating women with CKD, severe heart failure, severe hemodynamic disorders, cancer, and immune-related nephritis. Patients who did not cooperate were excluded. Sixty-five CKD patients were enrolled in the study, and 18 were eventually included because of the exclusion criteria or because they dropped out. Of the 47 patients, the number of patients with stage CKD1-4 was 13, 11, 11, and 12, respectively. The diagnostic criteria for all patients with CKD were based on the KDIGO guideline.[16] The estimated glomerular filtration rate (eGFR) was calculated using an epidemiological collaboration (CKD-EPI) equation.[17] This study was approved by the Ethics Research Society of the First Affiliated Hospital of Soochow University (no. 079). All participants signed an informed consent form. Our study was complied with the Declaration of Helsinki.

Laboratory Measurements

To collect plasma,3 mL of fasting venous blood was obtained in the early morning of the day after admission and was stored in an EDTA anticoagulant tube. The sample was allowed to stand at room temperature for 20 minutes and then was centrifuged for 10 minutes at 3000rpm and 4°C. 500ul of the supernatant was transferred into a 2mL cryovial and was freezed at −80°C. For urine collection, mid-morning urine was obtained on the day after admission, which was placed in a sterile EP tube, and it was centrifuged for 10 minutes at 3000 rpm and 4°C. 1 mL of the supernatant was transferred into a 2 mL cryovial and was freezed at −80°C. Biochemical and routine hematological indicators were measured using an OLYMPUS AU2700 automatic biochemical analyzer (OLYMPUS, Japan) and a Beckman LH750 automatic hematology analyzer (Beckman, USA).

Metabolomics Analysis

Data analysis preprocessing: 1. Metabolic analysis was performed on the processed plasma and urine samples, and primary mass spectrometry data was collected. The system's stability was ensured by randomly inserting quality control samples into the sequence. 2. The obtained raw data was converted into mzXML format using Proteowizard software (v3.0.8789). 3. The XCMS package of R (v3.3.2) was used for peaks identification, filtration, and alignment. 4. A data matrix was obtained, which included information such as mass to charge ratio, retention time, and intensity. 5. To facilitate comparison of data with different magnitudes, batch normalization of peak areas was performed on the data.

LC (Ultimate 3000, Thermo Fisher Scientific, Waltham, MA, USA) parameter settings: Chromatographic separation was accomplished in a Thermo Ultimate 3000 system equipped with an ACQUITY UPLC® HSS T3 (150×2.1 mm, 1.8 μm, Waters) column maintained at 40°C. The temperature of the autosampler was 8°C. Gradient elution of analytes was carried out with 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) or 5mM ammonium formate in water (C) and acetonitrile (D) at a flow rate of 0.25mL/min. Injection of 2μL of each sample was done after equilibration. An increasing linear gradient of solvent B (v/v) was used as follows: 0~1 min, 2% B/D; 1~9 min, 2%~50% B/D; 9~14 min, 50%~98% B/D; 14~15 min, 98% B/D; 15~15.5 min, 98%~2% B/D; 15.5~17 min, 2%B/D.

MS (Q Exactive, Thermo Fisher Scientific) parameter settings:

The ESI-MSn experiments were executed on the Thermo Q Exactive Focus mass spectrometer with the spray voltage of 3.8 kV and −2.5 kV in positive and negative modes, respectively. Sheath gas and auxiliary gas were set at 45 and 15 arbitrary units, respectively. The capillary temperature was 325 °C respectively. The Orbitrap analyzer scanned over a mass range of m/z 81–1000 for full scan at a mass resolution of 70,000. Data dependent acquisition (DDA) MS/MS experiments were performed with HCD scan. The normalized collision energy was 30 eV.

The LC-MS data analysis process involved several key steps: 1. Data preprocessing, which includes data format conversion, peak identification, filtering, alignment, and normalization. 2. Data inspection, where chromatograms are drawn and quality control is performed. 3. First-level analysis includes metabolite hierarchical clustering, sample dendrogram analysis, multivariate statistical analysis, and screening for differential metabolites. 4. Secondary analysis comprises identifying, statistically analyzing, hierarchically clustering, creating correlation heat maps, generating ROC curves, and analyzing metabolic pathways of differential metabolites.

Differential metabolite analysis was performed using the following steps: 1. Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) was generated to visually demonstrate the classification effect of the model in both positive and negative ion modes for the control group and the experimental group. 2. Differential metabolites between groups were identified using Variable Importance for the Projection (VIP) (VIP value ≥ 1.0 and $P < 0.05$) as the screening criterion. The significantly different metabolites were further analyzed by confirming their precise molecular weight and matching the fragmentation information obtained in MS/MS mode with databases such as HMDB, Metlin, massbank, LipidMaps, and mzclound to obtain accurate metabolite information. 3. Possible bioperturbed metabolic pathways were identified using the MetPA database, and the metabolic pathways of the metabolites were analyzed.

Statistical Analysis

SPSS version 22.0. was conducted for statistical analysis. Means ± SDs, medians, and interquartile ranges (IQRs) were expressed for normal and non-normal distribution data. In the case of non-normally distributed data, we initially applied a logarithmic transformation. If the data, after being transformed logarithmically, satisfies the criteria of normal distribution and homogeneity of variance, we will proceed with conducting a one-way analysis of variance. One-way analysis of variance was performed for variables with normal distribution and homogeneous variance. A rank-sum test was performed on variables that were not normally distributed. Spearman correlation analyses were performed. Binary and ordinal logistic regression analyses analyzed the independent relationships with CKD. The receiver operating characteristic (ROC) curve was used to determine the diagnostic accuracy, and the Youden index was calculated to obtain the optimal cutoff point of the ROC curve. $P < 0.05$ (*) and $P < 0.01$ (**) were considered statistically significant.

Results

Clinical Date

The normality and homogeneity of all the clinical variables in the control and CKD groups were assessed. Relative to all 47 CKD patients, the control group had higher levels of hemoglobin and albumin and lower levels of Ln (creatinine) and Ln (BUN) than the control group; CKD patients had lower hemoglobin and albumin levels but higher Ln(creatinine) and Ln(BUN) levels. ($P < 0.01$), respectively (Table 1).

ILA Screening

We performed non-targeted LC-MS analysis of the enrolled participants and metabolite hierarchical clustering analysis after quality control, then we use the OPLS-DA to observe the distribution of each sample in the mathematical model space. Based on the conditions of $P < 0.05$ and VIP≥1.0, we obtained 1509 and 1236 differential metabolites in positive and negative ion modes in plasma, respectively, and we obtained 2491 and 1539 in positive and negative ion modes in urine, respectively. According to the fragmentation information obtained from MS/MS mode, 147 differential metabolites in plasma and 238 differential metabolites in urine were matched in HMDB, Metlin, massbank, LipidMaps and mzclound databases (Figure 1).

Table 1 Clinical Characteristics of Control and CKD Groups

	Control	CKD stage 1	CKD stage 2	CKD stage 3	CKD stage 4	*P*
Gender (male/female)	10/20	5/8	7/4	6/5	7/5	
Age(years)	48±12	47±16	51±21	56±17	58±14	0.232
Uric acid(umol/L)	344.52±90.95	379.78±122.66	430.31±59.78	431.20±129.65	420.37±138.40	0.060
Hemoglobin(g/L)	140.00±11.43	128.46±17.69	126.64±11.42	121.91±12.85	111.25±17.93	<0.01
Ln(Creatinine) (umol/L)	4.14±0.19	4.00±0.23	4.54±0.22	4.93±0.21	5.45±0.15	<0.01
Ln(Blood urea nitrogen) (mmol/L)	1.55±0.22	1.52±0.32	1.82±0.29	2.20±0.28	2.51±0.36	<0.01
Ln(Triglyceride) (mmol/L)	0.28±0.53	0.47±0.68	0.36±0.40	0.47±0.26	0.56±0.44	0.473
Ln(BMI) (Kg /m^2)	3.16±0.15	3.18±0.13	3.14±0.13	3.21±0.09	3.18±0.11	0.755
Albumin (g/L)	48(46,49)	36(29,41)	40(37,43)	43(39,44)	41(38,43)	<0.01
Total cholesterol(mmol/L)	4.5(3.9,5.2)	5.2(4.1,5.6)	4.7(4.5,5.5)	4.5(4.2,5.1)	4.5(3.8,5.2)	0.419
Blood glucose(mmol/L)	4.9(4.6,5.2)	4.6(4.5,4.8)	4.6(4.1,4.9)	4.7(4.1,4.8)	4.6(4.3,5.0)	0.086

Abbreviation: CKD, chronic kidney disease.

In the positive and negative ion modes, we found significant differences in plasma and urine ILA between patients with CKD and controls. We performed a differential metabolite pathway analysis based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) and found that tryptophan metabolism was disordered, in which ILA was mainly involved (Figure 2).

Both plasma and urine ILA differed significantly between CKD patients and controls and showed an increasing trend with the progression of renal function (Figure 3).

Correlations of ILA with Clinical Indicators

Logistic regression analysis revealed a curvilinear correlation between plasma and urine ILA (r = 0.51, $P < 0.01$). We also investigated the correlation between plasma and urine ILA levels and other clinical indicators by Spearman correlation analyses. Plasma ILA was positively correlated with age, BMI, creatinine, BUN, triglycerides, and uric

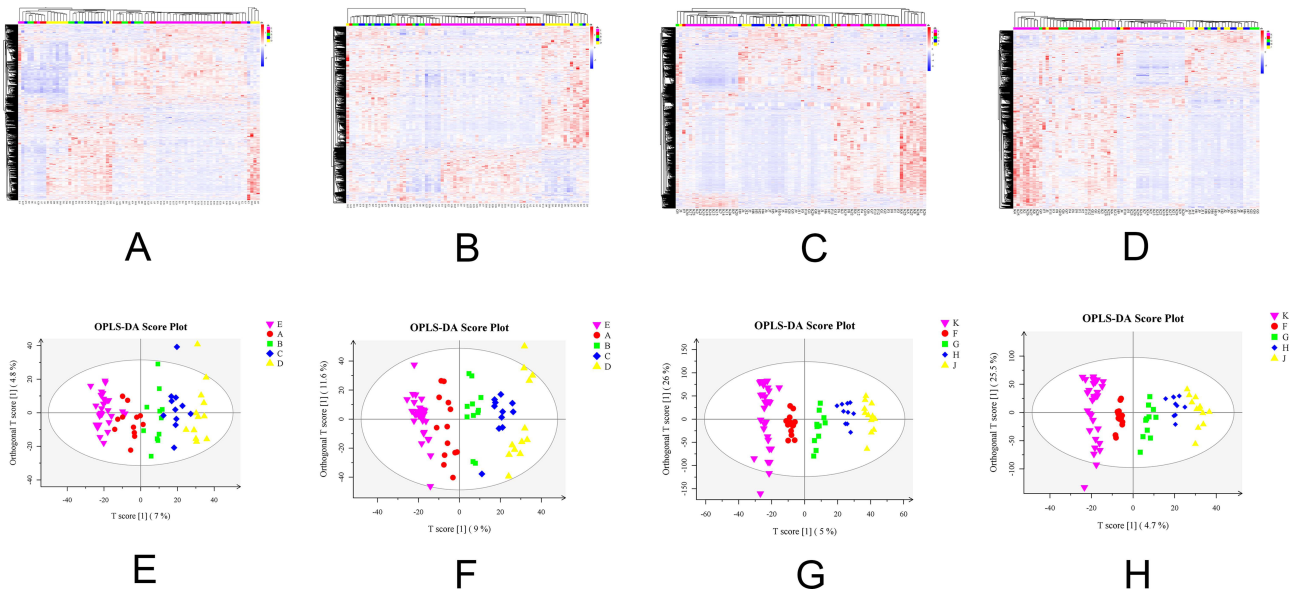

Figure 1 Metabolite hierarchical clustering and OPLS-DA analysis of Control and CKD groups.
Notes: (**A**) Heatmap of plasma metabolites hierarchical clustering analysis in positive ion mode; (**B**) Heatmap of plasma metabolites hierarchical clustering analysis in negative ion mode; (**C**) Heatmap of urine metabolites hierarchical clustering analysis in positive ion mode; (**D**) Heatmap of urine metabolites hierarchical clustering analysis in negative ion mode; (**E**) OPLS-DA analysis of plasma metabolites in positive ion mode; (**F**) OPLS-DA analysis of plasma metabolites in negative ion mode; (**G**) OPLS-DA analysis of urine metabolites in positive ion mode; (**H**) OPLS-DA analysis of urine metabolites in negative ion mode. The color purple was used to represent the control group, red was used for CKD stage 1 patients, green for CKD stage 2 patients, blue for CKD stage 3 patients, and yellow for CKD stage 4 patients.
Abbreviations: CKD, chronic kidney disease, OPLS-DA, Orthogonal Projections to Latent Structures Discriminant Analysis.

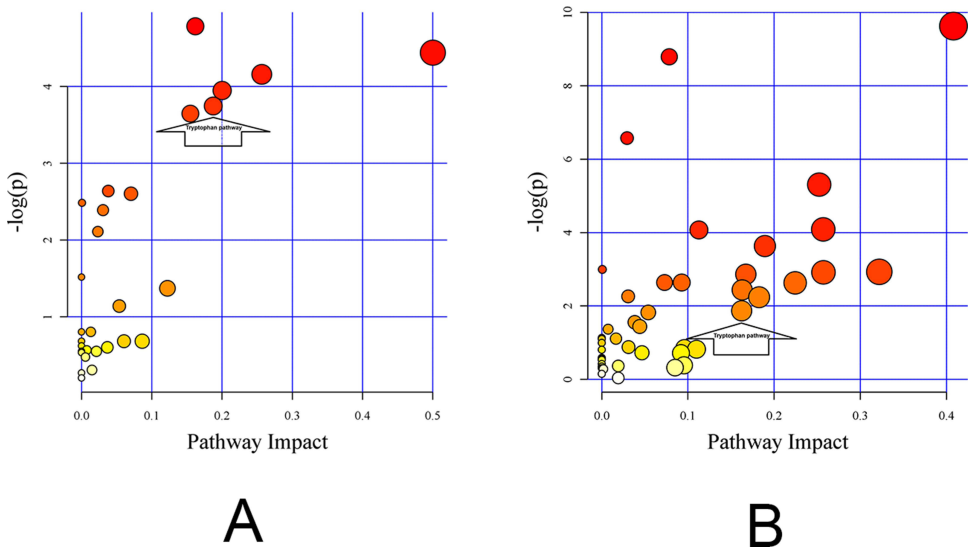

Figure 2 Differential metabolite pathway analysis of Control and CKD groups.
Notes: (**A** and **B**) Plasma and urine.
Abbreviation: CKD, chronic kidney disease.

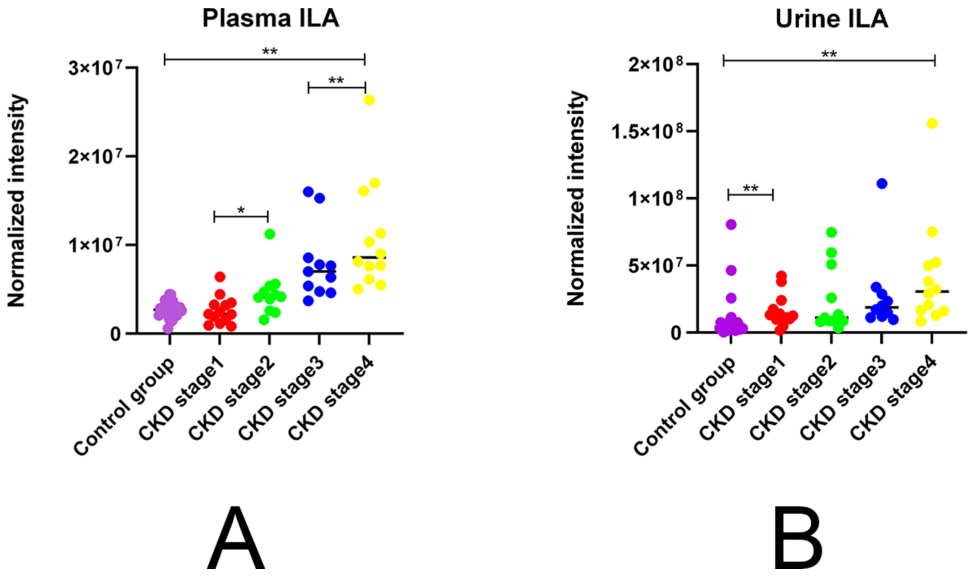

Figure 3 ILA of Control and CKD groups.
Notes: (**A**) Plasms ILA; (**B**) Urine ILA; *: $P < 0.05$; **: $P < 0.01$.
Abbreviations: CKD, chronic kidney disease; ILA, indole lactic acid.

acid (r = 0.40, 0.28, 0.78, 0.67, 0.37, 0.33, $P < 0.05$) and negatively correlated with hemoglobin (r = –0.28, $P < 0.05$). Urine ILA was positively correlated with age, creatinine, BUN, and uric acid (r = 0.48, 0.56, 0.54, 0.27, all $P < 0.01$) and negatively correlated with hemoglobin and albumin (r = –0.35, –0.51, $P < 0.01$) (Figure 4).

Logistic Regression Analysis of CKD-Related Factors

We performed binary logistic regression analysis to assess the relationship between plasma and urine ILA, and the associations between plasma and urine ILA and CKD were assessed using binomial logistic regression analysis. CKD was associated with ILA in the plasma and urine (OR = 5.20, 5.24, $P < 0.01$) before adjusting for confounders (Table 2).

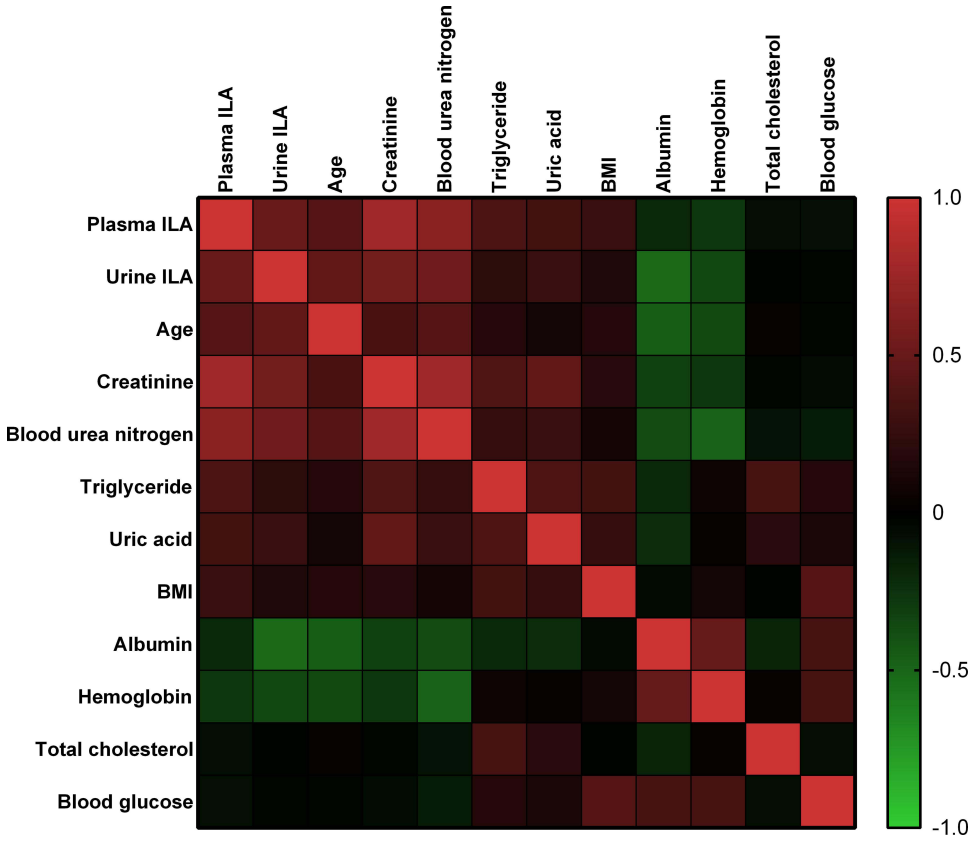

Figure 4 Relationship of plasma and urine ILA with different clinical indices.
Abbreviation: ILA, Indole lactic acid.

Considering the confounding factors of plasma ILA, urine ILA, hemoglobin, and hemoglobin, ordinal logistic regression showed that CKD was significantly associated with plasma ILA, urine ILA, BUN, and hemoglobin levels (OR = 4.49, 2.14, 1.43, 0.95, $P < 0.05$) (Table 3).

ILA in Plasma and Urine Predicted CKD

By comparing 47 patients with CKD with 30 controls, we performed receiver operating characteristic analysis to determine the plasma and urine ILA predictive of CKD. (Figure 5). The area under the curve (AUC) for plasma ILA was 0.755 (95% CI:0.666–0.883, $P < 0.01$), with an optimal cutoff value of 0.607, a sensitivity of 67.4%, and a specificity of 93.3%. The area under the curve (AUC) of urinary ILA was 0.869 (95% confidence interval [CI]:0.772–0.965, $P < 0.01$), with an optimal cutoff value of 0.735, a sensitivity of 93.5%, and a specificity of 80.0%. We conducted a Spearman rank analysis to examine the relationship between plasma and urine ILA levels and the stage of CKD (r=0.71, $P < 0.01$; r=0.65, $P < 0.01$), which revealed a positive correlation between plasma and urine ILA levels and the severity of CKD. Therefore, both plasma and urine ILA are reliable predictors of CKD.

Table 2 Binary Logistic Regression Analysis of ILA Independently Associated with CKD

	OR	*P*
Plasma ILA	5.20	< 0.01
Urine ILA	5.24	< 0.01

Abbreviations: CKD, chronic kidney disease; ILA: Indole lactic acid.

Table 3 Ordinal Logistic Regression Analysis of Risk Factors Independently Associated with CKD

	OR	P
Plasma ILA	4.49	< 0.01
Urine ILA	2.14	< 0.01
Blood urea nitrogen	1.43	< 0.01
hemoglobin	0.95	< 0.01

Abbreviations: CKD, chronic kidney disease; ILA: Indole lactic acid.

Association Between Inflammatory Markers and CKD

Neutrophil-to-Lymphocyte Ratio (NLR), Monocyte-to-Lymphocyte Ratio (MLR), and Hs-CRP levels were compared in patients with CKD (Figure 6). The results showed that the NLR, MLR, and Hs-CRP levels in the control group and patients with CKD showed a clear upward trend ($P < 0.05$).

Correlations of ILA with Inflammatory Indicators

Spearman correlation analysis indicated that plasma ILA was positively correlated with NLR, Hs-CPR, and MLR (r = 0.24, 0.56, and 0.37, $P < 0.05$). Urine ILA was positively correlated with NLR, Hs-CRP, and MLR (r = 0.40, 0.57, and 0.30; $P < 0.01$) (Figure 7).

Ordinal Logistic Regression Analysis of the Relationship Between Inflammation and CKD

We included NLR, MLR, Hs-CRP, plasma ILA, and urine ILA for Ordinal logistic regression. The results showed that CKD was associated with three inflammatory factors: plasma ILA, urine ILA, and Hs-CRP (OR = 5.92, 2.79, 2.45, $P < 0.01$) (Table 4).

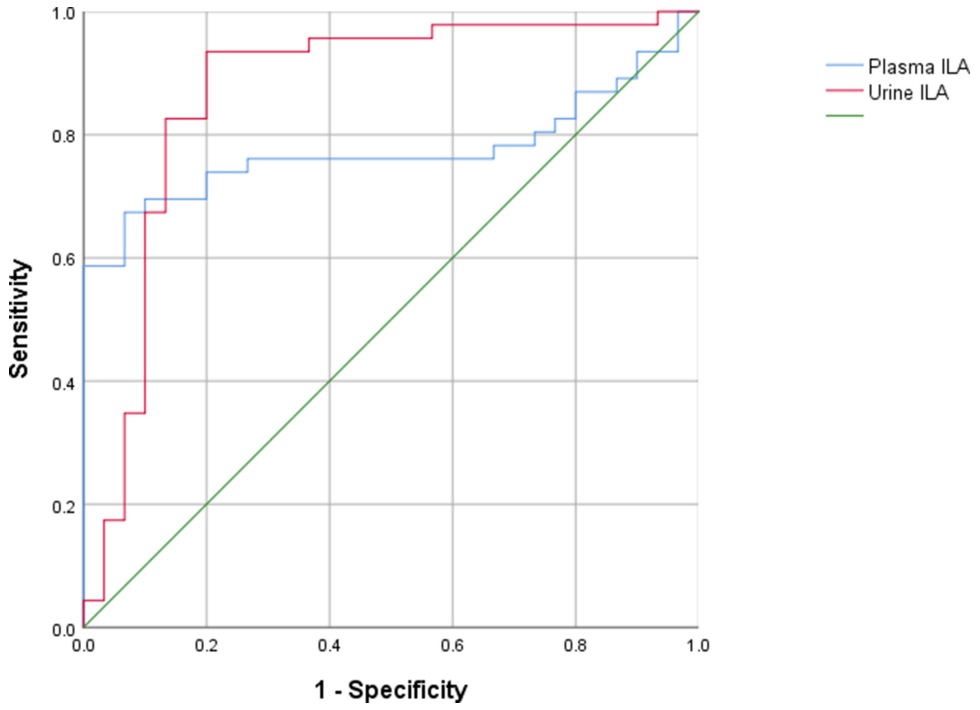

Figure 5 Receiver operating characteristic analysis for prediction of CKD based on plasma and urine ILA.
Abbreviations: CKD, chronic kidney disease; ILA: Indole lactic acid.

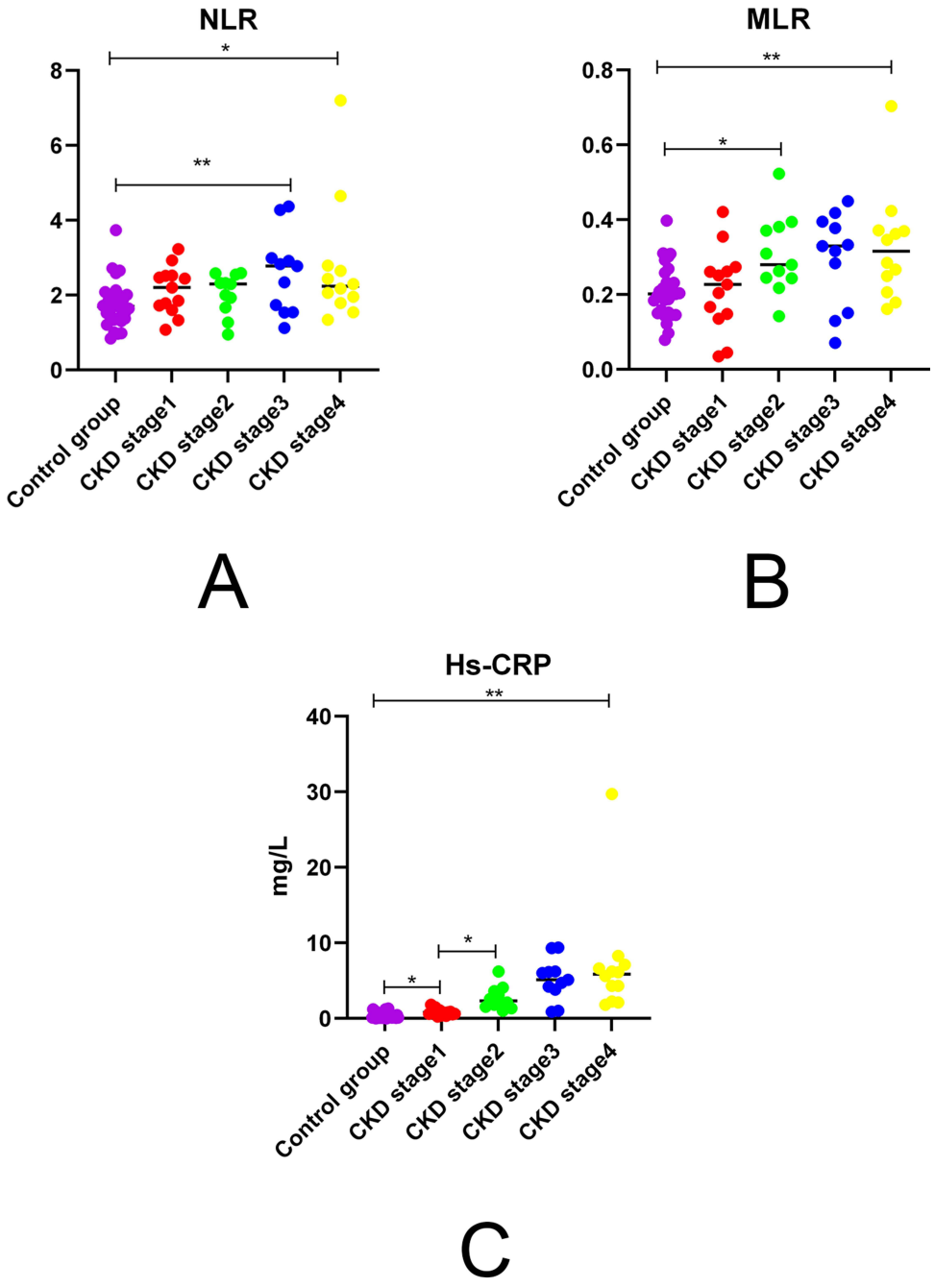

Figure 6 NLR, MLR, Hs-CRP: Changes of Inflammatory indicators in control and CKD group.
Notes: (**A**) Changes of NLR in control and CKD group; (**B**) Changes of MLR in control and CKD group; (**C**) Changes of Hs-CRP in control and CKD group; NLR: Neutrophil-to-Lymphocyte Ratio, MLR: Monocyte-to-Lymphocyte Ratio; *: $P < 0.05$; **: $P < 0.01$.
Abbreviations: CKD, chronic kidney disease; ILA: Indole lactic acid.

Discussion

We conducted a metabolomic analysis of non-dialysis CKD patients, aiming to clarify metabolic differences between non-dialysis patients with CKD and healthy people and further explore the relationship between metabolic differences, clinical indicators, and inflammation to provide a means for detecting, diagnosing, and treating CKD. Non-targeted LC-MS analysis was also performed. Quality assurance was performed based on quality control to remove feature peaks with poor repeatability in the quality control samples to obtain high-quality datasets. We performed a differential metabolite pathway analysis based

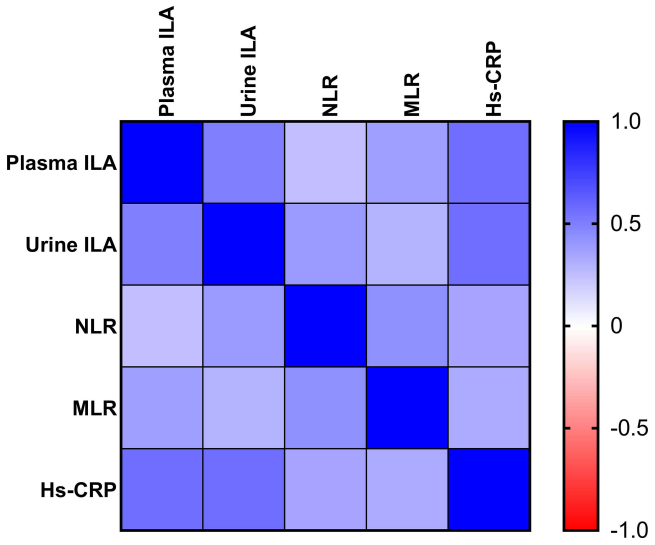

Figure 7 Correlation of Plasma and Urine ILA with Inflammatory indicators.

on KEGG and identified disorders of tryptophan metabolism involving ILA. Metabolite hierarchical clustering analysis was performed, and we found differential metabolite ILA in the plasma and urine of patients with CKD versus healthy people.

ILA are the downstream products of tryptophan metabolism. There is limited research on changes in blood ILA levels in patients with CKD. Previous studies have indicated that blood ILA levels increase as the eGFR decreases, consistent with our findings.[18–20] To the best of our knowledge, this is the first study to examine ILA levels in both the plasma and urine of patients with CKD. We compared the control group with all patients with CKD to show that CKD was associated with plasma and urine ILA (OR = 5.20, 5.24, $P < 0.01$). We analyzed the correlation between plasma and urine ILA levels and clinical indicators and identified relevant clinical indicators. Subsequently, we performed an ordered regression analysis, and the results showed that plasma and urine ILA (OR = 4.49, 2.14, $P < 0.01$) were still strongly associated with CKD. In addition, we performed an ROC analysis and found that plasma ILA (AUC=0.755, $P < 0.01$) and urine ILA (AUC=0.869, $P < 0.01$) for CKD showed good forecast performance. It is worth noting that urine ILA has a higher predictive value than plasma ILA, perhaps a more important concern for clinicians regarding changes in urine than in plasma. Most patients with stages 1 and 2 CKD are asymptomatic and have normal or mild renal function, whereas patients with stages 3 and 4 CKD have moderate-to-severe renal impairment. Early recognition and treatment of patients with early CKD can delay the progression to advanced renal failure and end-stage renal disease. We found significant differences in plasma ILA levels between healthy individuals and patients with CKD stages 1–4. This suggests that ILA should be focused on the early stages of CKD. Regrettably, our analysis did not reveal substantial disparities between

Table 4 Ordinal Logistic Regression Analysis of the Association of Inflammatory Factors with CKD

	OR	*P*
Plasma ILA	5.48	< 0.01
Urine ILA	2.85	< 0.01
Hs-CRP	2.45	< 0.01
NLR	0.92	0.82
MLR	7.41	0.47

Abbreviations: CKD, chronic kidney disease; ILA: Indole lactic acid; NLR: Neutrophil-to-Lymphocyte Ratio; MLR: Monocyte-to-Lymphocyte Ratio,

plasma ILA and urinary ILA in healthy individuals or patients with CKD stages 1–2, which may be related to smaller sample sizes or ILA in vivo binding and clearance mechanisms. We suggest that larger multicenter clinical studies be conducted to define specific reference values for adult ILA. Elevated plasma and urine ILA in patients with CKD suggest that altered ILA and abnormalities in tryptophan metabolic pathways may participate in the development of CKD. Our findings suggest that changes in plasma and urine ILA levels may be useful for identifying patients with non-dialysis CKD and monitoring CKD progression. We suggest that more studies examine changes in plasma and urine ILA simultaneously. In addition, because ILA, one of the receptors for AhR, is involved in regulating immunity, the level of ILA should be explained carefully to patients with CKD taking immunomodulatory drugs.

Lipid metabolism is disrupted in patients with CKD progression, which mainly involves elevated triglyceride.[21,22] This is closely associated with cardiovascular events in patients with CKD. Statins are commonly used clinically for lipid regulation and reducing the occurrence of CVD events.[23] Interestingly, Lee et al found that ILA reduces mice's liver fat accumulation and body weight.[24] We found a positive correlation between ILA and triglycerides. However, this correlation was not strong and may be related to mild inflammatory and oxidative stress in the early stages of CKD. Dietary and pharmacological interventions for the level of ILA in patients with early-stage CKD warrant further studies. Our study found that plasma and urine ILA were negatively correlated with hemoglobin levels in patients with CKD, suggesting that toxin accumulation may affect the anemia status of patients with CKD. After our ordered regression analysis, we found that, in addition to plasma and urine ILA, hemoglobin was also an independent factor in the occurrence of CKD. Hemoglobin is a protective factor against diseases, consistent with our clinical understanding.[25,26] Simultaneously, clinicians should be more careful about the relationship between anemia and urine toxins in patients with CKD.

ILA acts as a ligand of AhR, influencing inflammation and the immune response. In an in vivo experiment, ILA downregulated the transcription factor Thpok by activating AhR. This allows CD4 + T cells to be reprogrammed into CD4CD8αα T cells.[27] ILA upregulates immunoregulatory galectin-17 in Th1 and Th2 cells, which is vital in mediating the functional connection between beneficial microbes and immune regulation.[28] ILA has been found to preserve the integrity of the epithelial barrier by modulating the AHR/NRF3/NLRP2 pathway to upregulate tight junction proteins.[29] Moreover, ILA can attenuate NF-kB activation in macrophages and the expression of TNF-α and IL-8 induced by LPS.[30,31] Riazati et al discovered a positive correlation between plasma indole and CRP levels.[32] Our previous study showed that Hs-CRP levels were significantly altered early in patients with CKD. We observed a positive correlation between the ILA and Hs-CRP levels. NLR and MLR are readily available indicators in clinical patients and are currently used to evaluate the inflammatory state of diseases.[33,34] Lano et al found that the NLR was positively correlated with urine toxins in patients on dialysis.[35] A study based on CKD stages 1–3 showed that the NLR of patients with stage 3 CKD was higher than that of patients with stage 1 and 2 CKD.[36] Mureșan AV et al found that MLR predicted all-cause mortality and correlated with length of hospital stay and duration of dialysis in end-stage renal disease patients.[37] One study based on SARS patients hospitalized for COVID-19 showed that MLR can predict mortality in patients with CKD in this patient population.[38] NLR and MLR were significantly elevated in CKD stages 1–4 compared to healthy subjects in this study. NLR and MLR were positively correlated with plasma and urine ILA levels. Ordered regression analysis of plasma ILA, urine ILA, Hs-CRP, NLR, and MLR showed that plasma ILA, urine ILA, and Hs-CRP levels were independent factors for inflammation in CKD. The OR value was greater for plasma and urine ILA than for Hs-CRP. More attention should be paid to the role of ILA in developing oxidative stress and inflammation in CKD. In addition, sufficient attention should be paid to oxidative stress and inflammatory states in patients with stages 1–2. Breast milk and a diet rich in tryptophan can increase the ILA intake. Additionally, the expression of ILA can be affected by high salt intake.[39–42] It is important to note that excessive intake of ILA may impose a burden on the kidneys. Therefore, further research is necessary to determine the recommended ILA intake. There is limited research on the effects of ILA inhibition in patients with non-dialysis CKD. We recommend conducting more comprehensive studies on this topic in the future.

This study had some limitations. First, the sample size was small, and we did not include samples from patients with CKD stage 5 or those undergoing hemodialysis. Second, it was a single-center study that may limit the generalizability of the findings.

Conclusion

In this study, metabolomic analysis was performed on plasma and urine samples from non-dialysis CKD patients using LC-MS methods. Differential metabolic pathway analysis based on KEGG was conducted, revealing tryptophan metabolism disorders associated with ILA. Hierarchical clustering analysis of metabolites showed distinct levels of ILA in the plasma and urine of CKD patients compared to healthy individuals. This study represents the first simultaneous detection of ILA levels in both plasma and urine of chronic kidney disease patients. The findings suggest that plasma and urinary ILA could serve as potential biomarkers for CKD and inflammatory status.

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Disclosure

All authors report no conflicts of interest in this work.

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