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Clinical Center of Vojvodina, Center of Laboratory Medicine, Novi Sad[1]
University of Novi Sad, Faculty of Medicine Novi Sad,
Department of Pathophysiology and Laboratory Medicine[2]
Department of Pharmacy[3]
Center for Medical and Pharmaceutical Investigations and Quality Control[4]

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STATUS OF TRYPTOPHAN METABOLITES IN DIFFERENT STAGES OF CHRONIC KIDNEY DISEASE OF NON-DIABETC ETIOLOGY

STATUS METABOLITA TRIPTOFANA U RAZLIČITIM STADIJUMIMA HRONIČNE BUBREŽNE BOLESTI NEDIJABETESNE ETIOLOGIJE

Ivana ISAKOV[1], Velibor ČABARKAPA[1, 2], Branislava SRĐENOVIĆ ČONIĆ[3, 4], Nebojša KLADAR[3, 4], Branislava ILINČIĆ[1, 2] and Dragan BURIĆ[1, 2]

Summary

Introduction. Modification of tryptophan metabolism during the progression of chronic kidney disease may have significant pathophysiological consequences. The aim of this study was to investigate the status of metabolic products of tryptophan, indoxyl sulfate and kynurenine in different stages of chronic kidney disease. **Material and Methods.** In all participants included in the cross-sectional study (n = 66) with previously diagnosed chronic kidney disease, the parameters of renal function were measured: glomerular filtration rate using radionuclide plasma clearance with 99mTc-labelled diethylene triamine penta-acetate and effective renal plasma flow using 131I-labeled orthoiodohippuric acid. Plasma concentrations of indoxyl sulfate and kynurenine were measured by high-performance liquid chromatography. **Results.** A significant difference was observed in the concentrations of both metabolites between the observed groups (Group II – measured glomerular filtration rate - 15 - 60 ml/min/1.73 m²; n = 36 vs. Group I measured glomerular filtration rate > 60 l/min/1.73 m²; n = 26): indoxyl sulfate 1.07 ± 0.89 vs. 2.44 ± 4.05 μg/ml, p < 0.001; kynurenine 3.15 ± 0.22 vs. 3.21 ± 0.17 μg/ml, p < 0.05. The correlation was statistically significant between glomerular filtration rate and kynurenine – r = -0.38, p = 0.001 and indoxyl sulfate – r = 0.56, p ≤ 0.001; effective plasma renal flow and kynurenine – r = -0.33, p < 0.05 and indoxyl sulfate – r = 0.46, p ≤ 0.001. **Conclusion.** There is a significant difference in the plasma concentrations of indoxyl sulfate and kynurenine in the group of patients with glomerular filtration rate of 15 - 60 ml/min/1.73 m² compared to patients with glomerular filtration rate > 60 ml/min/1.73 m². In patients with chronic kidney disease, plasma concentrations of both metabolites of tryptophan are inversely correlated with the glomerular filtration rate and effective plasma renal flow.

Key words: Kidney Failure, Chronic; Tryptophan; Glomerular Filtration Rate; Chromatography, High Pressure Liquid; Biomarkers; Kynurenine; Indican

Sažetak

Uvod. Poremećaj metabolizma triptofana koji se javlja tokom progresije hronična bubrežne bolesti može imati značajne patofiziološke posledice. Cilj ove studije bio je ispitivanje statusa metabolite triptofana, indoksilsulfata i kinurenina u različitim stadijumima hronična bolesti bubrega. **Materijal i metode.** Svim pacijentima obuhvaćenim studijom preseka (n = 66) sa prethodno dijagnostikovanom hroničnom bolesti bubrega izmereni su parametri za procenu bubrežne funkcije: jačina glomerulske filtracije pomoću plazma klirensa radionuklida99mTc-dietilen triamin pentaacetata i efektivni bubrežni protok plazme pomoću 131 I – ortojodhipurne kiseline. Plazmatske koncentracije indoksilsulfata i kinurenina određene su metodom tečne hromatografije visokih performansi. **Rezultati.** Uočena je značajna razlika u koncentracijama oba metabolite između posmatranih grupa (Grupa II – mGFR – 15–60 ml/min/1,73 m²; n = 36) vs (Grupa I mGFR > 60 ml/min/1,73 m²; n = 26): indoksil sulfat 1,07 ± 0,89 vs 2,44 ± 4,05 μg/ml, p < 0,001; kinurenin 3,15 ± 0,22 vs 3,21 ± 0,17 μg/ml, p < 0,05. Korelacija je bila statistički značajna između jačine glomerulske filtracije i kinurenina – r = -0,38, p = 0,001 i indoksil sulfata r = 0,56, p = < 0,001; efektivni bubrežni protok plazme i kinurenin r = -0,33, p < 0,05 i indoksil sulfata – r = 0.46, p = < 0,001. **Zaključak.** Postoji značajna razlika u plazmatskim koncentracijama indoksilsulfata i kinurenina u grupi pacijenata sa jačinom glomerulske filtracije 15–60 ml/min/1,73 m² u poređenju sa pacijentima sa jačinom glomerulske filtracije > 60 ml/min/1,73 m². Kod pacijenata sa hroničnom bolesti bubrega plazmatske koncnetracije oba metabolita triptofana su u obrnutoj korelaciji sa jačinom glomerulske filtracije i efektivnim bubrežnim protokom plazme.

Ključne reči: hronična bubrežna bolest; triptofan; jačina glomerularne filtracije; tečna hromatografija visokih performansi; biomarkeri; kinurenin; indoksil

Corresponding Author: Dr Ivana Isakov, Klinički centar Vojvodine, Centar za laboratorijsku medicinu, 21000 Novi Sad, Hajduk Veljkova 1–7, E-mail: ivana.isakov@kcv.rs, velibor.cabarkapa@mf.uns.ac.rs

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Abbreviations

CKD	– chronic kidney disease
KYN	– kynurenine
GFR	– glomerular filtration rate
IS	– indoxyl sulfate
OAT	– organic anion transporter
CCV	– Clinical Center of Vojvodina
M	– male
F	– female
BMI	– body mass index
FMU	– first morning urine
DTPA	– diethylene triamine penta-acetate
ERPF	– effective renal plasma flow
CysC	– Cystatin C
HPLC	– high-performance liquid chromatography
WHR	– waist to hip ratio
HDL-c	– high density lipoprotein cholesterol
LDL-c	– low density lipoprotein cholesterol
CVD	– cardiovascular disease

Introduction

Chronic kidney disease (CKD) includes a heterogeneous group of kidney structure and function disorders that affects about 7% of the adult population over 30 years of age. It has been estimated that the occurrence of moderate forms of kidney function disorders in people over the age of 65 is present in as many as 30% of the population. Renal damage leads to a decline in the excretory, endocrine and metabolic functions of the kidneys [1]. Regardless of the underlying etiology, CKD is a slowly progressive disease leading to irreversible nephron loss and terminal renal disease followed by dialysis [2].

Various substances that are normally excreted by the kidneys accumulate in patients with CKD, which negatively affects many biological functions and contributes to the development of uremic syndrome. It is of great importance that the intestinal microflora is significantly altered due to the progression of CKD. Fermentation of proteins and amino acids by certain intestinal bacteria results in the formation of various metabolites that are absorbed into the circulation and retained in the body [3–7]. Namely, with the progression of CKD, especially in uremia, there is a breakdown of the intestinal epithelial 'tight junction' barriers, which affects the composition and metabolic activity of the intestinal microflora, leading to an increase in the production of harmful substances (toxins) that enter the circulation and cause systemic inflammation [8]. In advanced stages of CKD, especially in terminal forms of the disease, the diversity of intestinal flora is reduced and the presence of aerobic bacteria, such as Enterobacter and Escherichia coli, is increased, leading to an imbalance in the intestinal ecosystem and finally, the production of protein-binding uremic toxins through the proteolysis of undigested proteins retained in the intestines [9].

Tryptophan is an essential amino acid that must be provided through the diet for the needs of the body. Due to the complexity of tryptophan metabolic pathways, different properties of tryptophan metabolic products are associated with different pathophysiological conditions [10, 11]. Approximately one third of the total tryptophan in the body comes from food, and the rest from protein degradation. Tryptophan metabolism involves three metabolic pathways in the gut: 1) the kynurenine (KYN) pathway, in epithelial and immune cells, 2) serotonin pathway, in enterochromic cells, and 3) indole pathway, associated with the intestinal microflora [12]. Over 95% of absorbed tryptophan is catabolized via the kynurenine pathway, while only 1 – 2% via the serotonin and 2 – 3% via the indole pathways [13].

The KYN is the first stable product and the key point of the kynurenine pathway. Three major transformation pathways diverge from KYN: a) deamination to kynurenic acid b) degradation to anthranilic acid, and c) conversion to 3-hydroxykynurenine [14]. The KYN and its metabolites are excreted in the urine reaching the urine during the glomerular filtration process [15].

Indoxyl sulfate (IS) is produced when dietary tryptophan is metabolized into indole. Indole is absorbed by the intestines and reaches the liver through circulation. After metabolic reactions in the liver, indole becomes IS and re-enters the blood circulation. In normal physiological conditions, IS enters the cells of the proximal tubules via organic anion transporters (OAT): OAT1 and OAT3 localized in basolateral membrane of proximal tubule cells, and then it is excreted from the cells via OAT4 localized in the apical membrane of renal tubular cells [16].

Modification of tryptophan metabolism and accumulation of certain toxic metabolites in the body is one of the most important factors for the development of CKD symptoms such as neurological disorders, bone metabolism disorders, impaired lipid metabolism, endothelial dysfunction leading to atherosclerosis, hypercoagulability, and blood vessel calcification, which is directly related to a higher prevalence of cardiovascular incidents [17–21].

The aim of this study was to examine the status of tryptophan metabolic products, IS and KYN, in patients with different stages of CKD of non-diabetic etiology.

Material and Methods

This cross-sectional study was conducted at the Clinical Center of Vojvodina (CCV) in Novi Sad in the period March – October, 2021. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the CCV; informed consent for participation in the study was obtained from each participant.

The study included a total of 66 participants with previously diagnosed CKD who were being treated at the CCV. Considering various pathophysiological mechanisms that led to the development of CKD, an important factor in the selection of participants was information about previously diagnosed diabetes mel-

litus. The participants were divided into two groups based on the values of glomerular filtration rate (GFR). The first group included 26 participants: male (M) = 11, female (F) = 15 with GFR > 60 ml/min/1.73 m², and the second group included 40 participants: M = 22, F = 18, with GFR between 15 – 60 ml/min/1.73 m².

The exclusion criteria from the study were: a) patients under 20 years of age, b) patients treated for diabetes, c) patients with a kidney transplant, d) patients with GFR < 15 ml/min/1.73 m², e) patients with cardiovascular or cerebrovascular accidents in the previous 3 months, f) patients with inflammatory bowel disease, g) patients with impaired liver function, h) patients with a malignant disease.

All anthropometric measurements were performed with participants lightly dressed and barefoot. Body height was measured with a Harpenden anthropometer (Holtain Ltd, Croswell, UK) with 0.1 cm accuracy, while body weight was measured using an electronic scale with 0.1 kg accuracy. The body mass index (BMI) was obtained by calculation according to the formula: body weight in kilograms divided by the square of body height expressed in meters. Waist and hip circumferences were measured using a flexible measuring tape with a precision of 0.1 cm in a standing position. Waist circumference was obtained by measuring at the level between the lowest point of the costal arch and the upper border of the iliac crest, while the hip circumference was measured around the widest part of the thigh.

Blood pressure was measured using a mercury manometer by the Riva Rocci auscultatory method. Blood samples for analysis were taken from the cubital vein in the morning after 12-hour overnight fast, and the urine sample for processing was the first morning urine (FMU). The samples were taken for processing immediately after sampling.

In order to assess kidney function as accurately as possible, the following parameters were assessed:

– The GFR was measured using a single injection 99mTc-diethylene triamine penta-acetate (DTPA) clearance with a two point sampling approach at 180 min and 240 min post injection (37 MBq) according to the method described by Russell et al.

– Effective renal plasma flow (ERPF) was determined by the isotopic clearance of ^{131}I labeled orthoiodohippuric acid (hippuran) from two blood samples, collected after 20 and 30 minutes, using the Blaufox method. The ERPF values were expressed in ml/min/1.73 m^2 [22].

For the quality control of the radiochemical purity of isotopes (> 95%), paper chromatography was used. For accurate measurement of the sample radioactivity, gamma counter with NaI (Tl) crystals (Captus 3000 by Capintec, USA) was used.

– Cystatin C (CysC) was measured by immunoturbidimetric method using a biochemical analyzer ADVIA 1800, and GFR was calculated using formulas:

If the CysC ≤ 0.8: eGFR (mL/min/1.73 m^2) = 133 x (CysC/0.8)-0.499 x 0.996 years (x 0.932 if female).

If the CysC > 0.8: eGFR (mL/min/1.73 m^2) = 133 x (CysC/0.8)-1.3289 x 0.996 years (x 0.932 if female) [23].

– Urea, creatinine and uric acid were determined by standard biochemical methods using a biochemical analyzer ADVIA 1800 with Siemens commercial kits (Siemens kits, Erlangen, Germany);

– Albumin and creatinine concentrations in FMU sample were determined by immunoturbidimetry method using an Alinity C biochemical analyzer and commercial Abbott kits (Abbott kits, Wiesbaden, Germany). The albumin-creatinine ratio index was calculated as the quotient of the obtained values.

Plasma and FMU samples, i.e., prepared diluted solutions for the determination of IS and KYN, were stored frozen at -20 °C until the moment of analysis, for a maximum of six weeks. The IS and KYN in human plasma and urine samples were measured by high-performance liquid chromatography (HPLC) using an Agilent HPLC 1100 system equipped with a diode array and fluorescent detectors (Agilent Technologies, Santa Clara, California, USA). Separation of analytes was done using Nucleosil RP C18 column (250 mm × 4.6 mm, 5 μm particle size). The quantification included isocratic elution at 1 mL/min flow of a mobile phase consisting of 95% (v/v) acetonitrile and 5% (v/v) acetate buffer (15 mM, pH = 4.5) at 25 °C and subsequent fluorescent detection for IS and ultraviolet (UV) detection at 225 nm for KYN. Prior to sample analysis, the corresponding calibration curves for IS and KYN were obtained under the previously stated experimental conditions, based on which the analytical method was validated.

The sample (human plasma and FMU) preparation included mixing 300 μL of sample and 900 μL of formic acid in acetonitrile (1%, v/v), centrifugation at 3500 rpm for 5 minutes and filtration of the obtained supernatant in the chromatography tube. The injection volume was 5 μL [24, 25].

Complete blood count was performed using a SYSMEX XN hematology counter with Siemens commercial kits (Siemens Health Care, Erlangen, Germany). Laboratory parameters (glucose, lipid status, apolipoprotein AI, apolipoprotein B, vitamin B12, folic acid) were determined by standard biochemical methods using the biochemical analyzer ADVIA 1800 with Siemens commercial kits (Siemens kits, Erlangen, Germany), while insulin was processed using an Alinity analyzer with Abbott commercial kits (Abbott kits, Wiesbaden, Germany).

Statistical analysis was done using the statistical program Statistica 20 (StatSoftInc, Tulsa, OK, USA). The data are shown by mean values ± standard deviation (SD). The F- and T-tests were used to determine statistically significant differences between groups of participants, while the χ^2 test was used for categorical data. Linear regression analysis was used for determining the correlation between different parameters. Values of $p < 0.05$ were considered statistically significant.

Results

A comparison of general characteristics between the two examined groups is given in **Table 1.** The average age was significantly lower in Group I than

Table 1. Characteristics of examinees with different stages of chronic kidney disease
Tabela 1. *Karakteristike ispitanika u različitim stadijumima hronične bubrežne bolesti*

Parameter *Parametar*	Group I/*I grupa* (N/*Br.* = 26) GFR > 60 ml/min *JGF > 60 ml/min*	Group II/ *II grupa* (N/*Br.* = 40) GFR > 60 ml/min *JGF < 60 ml/min*	p-value *p-vrednost*
Gender (male/female)/*Pol (muški/ženski)*	11/15	22/11	0.31
Age (years)/*Starost (godine)*	45.57 ± 16.36	60.18 ± 11.52	***< 0.001***
BMI (kg/m²)/*Indeks telesne mase (kg/m²)*	26.77 ± 3.69	28.35 ± 4.51	0.19
Waist circumference (cm)/*Obim struka (cm)*	91.81 ± 10.44	99.05 ± 13.72	***< 0.05***
WHR/*Odnos struk–kuk*	0.87 ± 0.08	0.93 ± 0.12	***< 0.05***
Hypertension (yes/no)/*Hipertenzija (da/ne)*	12/14	34/6	***0.05***
Systolic blood pressure (mmHg)/*Sistolni krvni pritisak (mmHg)*	132.61 ± 21.56	133.70 ± 26.89	0.86
Diastolic blood pressure (mmHg)/*Dijastolni krvni pritisak (mmHg)*	80.0 ± 11.7	80.0 ± 10.2	0.93
Glucose (mmol/L)/*Glukoza (mmol/L)*	5.28 ± 0.75	5.92 ± 1.71	0.07
HDL cholesterol (mmol/L)/*HDL holesterol (mmol/L)*	1.42 ± 0.45	1.22 ± 0.30	***< 0.05***
LDL cholesterol (mmol/L)/*LDL holesterol (mmol/L)*	3.35 ± 1.60	2.97 ± 0.82	0.21
Triglycerides (mmol/L)/*Trigliceridi (mmol/L)*	1.44 ± 1.00	1.71 ± 1.00	0.27
Apolipoprotein A1 (g/L)/*Apolipoprotein A1 (g/L)*	1.54 ± 0.35	1.42 ± 0.25	0.11
Apolipoprotein B (g/L)/*Apolipoprotein B (g/L)*	1.05 ± 0.40	1.01 ± 0.21	0.56
Insulin (mIU/L)/*Insulin (mIU/L)*	17.44 ± 13.72	23.74 ± 21.14	0.18
Folic Acid (nmol/L)/*Folna kiselina (nmol/L)*	12.13 ± 6.05	13.46 ± 9.02	0.51
Vitamin B12 (pmol/L)/*Vitamin B12 (pmol/L)*	238.07 ± 107.93	316.55 ± 340.49	0.26

Legend/*Legenda*: GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije;* BMI – body mass index/*indeks telesne mase*; WHR – waist to hip ratio/*Odnos struk/kuk*; HDL – high density lipoprotein/*lipoprotein visoke gustine*; LDL – low density lipoprotein/*lipoprotein niske gustine*

in Group II (45.6 ± 16.4 vs. 60.2 ± 11.5 years, p < 0.001). Among Group I participants, waist size and waist to hip ratio (WHR) were significantly lower compared to Group II participants (91.8 ± 10.4 vs. 99.1 ± 13.7 cm, p = 0.03; 0.9 ± 0.1 vs. 0.9 ± 0.1, p < 0.01). There was no significant difference in gender, BMI or in the measured blood pressure values (p = 0.31, p = 0.19, p = 0.93). As a comorbidity, hypertension was more common in Group II patients (12/14 vs. 34/6, p = 0.049). Statistically significant differences were observed between HDL cholesterol values, which were lower in Group II participants (1.42 ± 0.45 vs. 1.22 ± 0.30 mmol/L, p < 0.05).

The parameters used to assess the state of renal function are showed in **Table 2.** All the observed parameters, as expected, showed a statistically significant difference between the two groups (p < 0.001), except for albumin in FMU (p = 0.39).

Table 2. Parameters for the assessment of kidney function
Tabela 2. *Parametri za procenu bubrežne funkcije*

Parameter *Parametar*	Group I/*I grupa* (N/*Br.* = 26) GFR > 60 ml/min *JGF > 60 ml/min*	Group II/ *II grupa* (N/*Br.* = 40) GFR > 60 ml/min *JGF < 60 ml/min*	p-value *p-vrednost*
Urea (mmol/L)/*Urea (mmol/L)*	5.23 ± 1.28	9.52 ± 3.78	***< 0.001***
Creatinine (mmol/L)/*Kreatinin (mmol/L)*	80.53 ± 18.26	128.00 ± 57.54	***0.001***
mGFR – DTPA (ml/min/1.73 m²)/*JGF – DTPA (ml/min/1,73 m²)*	79.7 ± 15.0	39.7 ± 11.8	***< 0.001***
ERPF (ml/min)/*EBPP (ml/min)*	421.9 ± 90.3	250.4 ± 62.6	***0.001***
Cystatine C (mg/L)/*Cistatin C (mg/L)*	1.02 ± 0.19	1.75 ± 0.65	***< 0.001***
GFR Cystatine C/*JGF Cistatin C*	80.76 ± 21.38	42.40 ± 18.06	***< 0.001***
Creatinine FMU (mmol/L)/*Kreatinin PJU (mmol/L)*	7.88 ± 6.34	5.49 ± 2.86	***0.04***
Albumine FMU (g/L)/*Albumin PJU (g/L)*	105.33 ± 276.61	189.95 ± 452.04	0.39
Albumine/Creatinine Ratio (FMU)/*Albumin/kreatinin odnos (PJU)*	24.73 ± 69.46	49.36 ± 134.79	0.39

Legend/*Legenda*: GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije*; ERPF – effective renal plasma flow/*EBPP – efektivni bubrežni protok plazme*; FMU – first morning urine/*PJU – prvi jutarnji urin; DTPA – dietilen triamin penta acetat*

Table 3. Concentration of indoxyle sulfate and kynurenine in plasma and first morning urine sample
Tabela 3. *Koncentracije indoksil sulfata i kinurenina u plazmi i prvom jutarnjem urinu*

Variables *Varijable*	Group I/*I grupa* (N/*Br.* = 26) GFR > 60 ml/min *JGF > 60 ml/min*	Group II/*II grupa* (N/*Br.* = 40) GFR > 60 ml/min *JGF < 60 ml/min*	p–value *p–vrednost*
Indoxyl sulfate – p (μg/ml)/*Indoksil sulfat – p (μg/ml)*	1.07 ± 0.89	2.44 ± 4.05	***< 0.001***
Indoxyle sulfate FMU (μg/ml)/*Indoksil sulfat PJU (μg/ml)*	16.93 ± 34.80	22.98 ± 50.76	0.655
Kynirenine – p (μg/ml)/*Kinurenin – p (μg/ml)*	3.15 ± 0.22	3.21 ± 0.17	***< 0.05***
Kynurenine FMU (μg/ml)/*Kinurenin PJU (μg/ml)*	2.91 ± 0.49	2.85 ± 0.27	0.906

Legend/*Legenda*: GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije*; p – plasma/*plazma*; FMU – first morning urine/*PJU – prvi jutranji urin*

Table 4. Correlation analysis of indoxyle sulfate and kynurenine concentrations with the selected parameters
Tabela 4. *Korelaciona analiza koncentracije indoksil sulfata i kinurenina sa selektovanim parametrima*

	Kynurenine *Kinurenin*		Indoxyl sulfate *Indoksil sulfat*	
Parameter *Parametar*	r	p–value *p–vrednost*	r	p–value *p–vrednost*
Age/*Starost*	0.02	0.81	0.1	0.41
Waist circumference/*Obim struka*	0.17	0.90	0.29	***0.01***
WHR/*Odnos struk/kuk*	0.12	0.33	0.27	***0.02***
BMI (kg/m²)/*Indeks telesne mase (kg/m²)*	0.18	0.08	0.39	***< 0.001***
Total cholesterol (mmol/L)/*Ukupni holesterol (mmol/L)*	-0.17	0.16	0.17	0.16
HDL cholesterol (mmol/L)/*HDL holesterol (mmol/L)*	-0.19	0.12	0.19	0.12
LDL cholesterol (mmol/L)/*LDL holesterol (mmol/L)*	-0.20	0.09	0.12	0.35
Triglycerides (mmol/L)/*Trigliceridi (mmol/L)*	0.16	0.21	0.05	0.66
Apolipoprotein A I (g/L)/*Apolipoprotein A I (g/L)*	-0.07	0.55	0.21	0.08
Apolipoprotein B (g/L)/*Apolipoprotein B (g/L)*	-0.12	0.30	0.12	0.34
Urea (mmol/L)/*Urea (mmol/L)*	0.39	***0.001***	0.70	***< 0.001***
Creatinine (mmol/L)/*Kreatinin (mmol/L)*	0.28	***< 0.05***	0.38	***< 0.001***
mGFR – DTPA (ml/min/1.73 m²)/*JGF – DTPA (ml/min/1,73 m²)*	-0.38	***0.001***	-0.56	***< 0.001***
ERBF (ml/min)/*EBPP (ml/min)*	-0.33	***< 0.05***	-0.46	***< 0.001***
Cystatine C (mg/L)/*Cistatin C (mg/L)*	0.30	***< 0.05***	0.69	***< 0.001***
GFR Cystatine C/*JGF Cistatin C*	-0.27	***< 0.05***	-0.54	***< 0.001***
Albumine/Creatinine Ratio (FMU)/*Albumin/Kreatinin odnos (PJU)*	0.15	***0.24***	0.25	***< 0.05***

Legend/*Legenda:* WHR – waist to hip ratio/*odnos struk/kuk*; HDL – high density lipoprotein/*lipoprotein velike gustine*; LDL – low density lipoprotein/*lipoprotein niske gustine*; GFR – glomerular filtration rate/*JGF – jačina glomerulske filtracije*; ERBF – effective renal blood flow/*EBPP – efektivni bubrežni protok plazme*; FMU – first morning urine/*PJU – prvi jutarnji urin*

The comparison of the measured concentrations of IS and KYN in plasma, showed higher plasma concentrations for both metabolites in Group II (IS 1.07 ± 0.89 vs. 2.44 ± 4.05 μg/ml, $p < 0.001$; KYN 3.15 ± 0.22 vs. 3.21 ± 0.17 μg/ml, $p < 0.05$), while the differences in FMU concentrations were insignificant (**Table 3**).

The correlation of metabolites with selected traits is shown in **Table 4.** Both metabolites showed a significant correlation with the parameters that are markers of renal function preservation. The KYN had a moderate correlation with urea, creatinine, eGFR - DTPA, ERPF and CysC ($p < 0.05$), while IS showed a high correlation with the same parameters ($p < 0.001$).

Discussion

Examination of the status of tryptophan metabolites, IS and KYN, in patients with different stages of CKD, showed that plasma concentrations of both metabolites were statistically significantly higher in CKD participants with GFR between 15 – 60 ml/min/1.73 m², compared to participants with GFR higher than 60 ml/min/1.73 m² (IS $p < 0.001$; KYN $p < 0.05$).

Two main causes are generally mentioned as the reasons for the increase in the level of the mentioned metabolites. Firstly, the increased immune activity leads to increased levels of proinflammatory factors, resulting in an increased activity of KYN pathway enzymes, and secondly, a decrease in the renal excretion

of these metabolites due to CKD related impaired renal function, leads to their accumulation in circulation and tissues [1, 26]. Another contributing factor to the altered tryptophan metabolism in CKD is the breakdown of intestinal 'tight junction' barriers, which affects the metabolic activity of the intestinal microflora [8].

The impaired tryptophan metabolism and the consequent accumulation of these metabolites are associated with a number of pathophysiological mechanisms in patients with CKD.

One of the mechanisms refers to the association of toxins produced by tryptophan fermentation and the occurrence of cardiovascular diseases (CVDs) in patients with CKD [19, 27]. Namely, tryptophan metabolites, formed mainly within the indole and KYN pathways, have pro-oxidative, pro-inflammatory, pro-coagulant and pro-apoptotic effects [27].

As a comorbidity, hypertension was, as expected, more common in patients with greater renal impairment. The effect of KYN and its metabolites on blood pressure has not been fully elucidated, because many studies suggest that certain metabolites play contradictory roles in vascular pressure [28, 29]. There are few studies on this topic, and some of them prove that certain metabolites of the KYN pathway may also be part of antihypertensive compensatory mechanisms. Hence, this issue requires further research so that the impact of changes in KYN pathway activity on the development of hypertension can be better understood.

Based on the available data related to our study groups, which refer to the health condition of participants, we noticed some differences in the number of antihypertensive drugs used by the examinees. In Group I (26 participants), 11 patients were without any antihypertnesive therapy, while the rest (n = 15) used 2 drugs on average, while in Group II (40 participants), only 2 patients were without any antihpertensive therapy, and more than half (n = 21) used 3 or more drugs as part of therapeutic protocols.

A correlation analysis showed that both metabolites of tryptophan significantly correlated with the renal function assessment parameters. The IS showed a high correlation with urea, creatinine, DTPA, ERBF, CysC ($r = 0.7$, $r = 0.38$, $r = -0.56$, $r = -0.46$, $r = 0.69$; $p < 0.001$), while KYN had a moderate correlation with the same parameters ($r = 0.39$, $r = 0.28$, $r = -0.38$, $r = -0.33$, $r = 0.30$; $p < 0.05$).

Harmful effects of tryptophan metabolites have been described in some earlier studies. The KYN metabolites can affect the proliferation rate of mesangial cells as well as the level of gene expression in these cells. In addition, elevated levels of KYN and its metabolites are associated with the progression of renal failure and glomerular fibrosis [16]. Also, IS significantly accumulates in the kidneys, inducing inflammatory reactions and enhancing oxidative stress, accelerating glomerular sclerosis and interstitial fibrosis, thus aggravating the decline of renal function [30]. It has been shown that IS specifically has a direct cytotoxic effect on renal tubular epithelial cells; after its cellular uptake by organic anion transporter, IS induces tubular cell necrosis [15]. Together, these mechanisms may contribute to the progression of CVDs and CKD [31].

Our study revealed that waist circumference (91.81 ± 10.44 vs. 99.05 ± 13.72 cm, $p = 0.03$) and WHR (0.87 ± 0.078 vs. 0.93 ± 0.12, $p = 0.01$) were significantly higher in the group with GFR 15 – 60 ml/min/1.73m^2, while the BMI value did not differ significantly between the examined groups. Our data are in line with the results of other authors [32] who emphasize WHR as one of the most important predictive factors for the development of CKD compared to other indices, pointing out that fat redistribution is important for the development of CKD, and not obesity per se.

A correlation analysis of IS and KYN showed a moderate correlation of IS with waist circumference ($r = 0.3$, $p = 0.01$), WHR ($r = 0.27$, $p = 0.02$) and BMI ($r = 0.39$, $p < 0.001$).

Of the metabolic parameters included in the study, only the level of HDL-c showed a significant difference between subjects of Group I and II, whereas lower values were observed in Group II participants (1.42 ± 0.45 vs. 1.22 ± 0.30 mmol/L, $p = 0.03$).

Reduced renal function is known to be associated with modification of lipoprotein structure and lipoprotein metabolism disorders [33–35]. According to some studies, the progressive loss of renal function is associated with increased concentration of IS and a decreased level of HDL-c [36] and a study by Li Wang et al. showed that there is an association between IS and HDL-c levels regardless of renal impairment, i.e., that IS is an independent risk factor for low HDL-c concentrations [37]. A correlating analysis of our results revealed that IS and KYN did not show a significant association with HDL-c values in the participants included in the study.

Conclusion

Based on the results of this study, it can be concluded that there is a significant difference in the concentrations of indoxyl sulfate and kynurenine in relation to different stages of chronic kidney disease, and that it is inversely correlated with measured glomerular filtration rate and measured effective renal plasma flow. Considering the numerous harmful effects of elevated plasma concentrations of indoxyl sulfate and kynurenine, further research is needed for a more comprehensive consideration of all pathophysiological mechanisms of chronic kidney disease involved in the dysregulation of tryptophan metabolism, as well as for finding an appropriate therapeutic model whose timely application would potentially reduce the aforementioned adverse effects, that is, slow down their clinical manifestation.

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