Evaluation of plasma malondialdehyde concentration and kynurenine/ tryptophan ratio in patients with stage 3-4 chronic kidney disease

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Abstract

Background: Chronic kidney disease (CKD) is increasingly recognized as a major health problem worldwide. This disease is associated with oxidative stress, which can generate the inflammatory process and promote renal injury progression. **Objectives**: (1) To evaluate the malondialdehyde concentration and kynurenine and tryptophan ratio for differences between CKD patients and healthy controls. (2) To analyze the relationship and correlation between these biomarker indexes and some risk factors of CKD. **Materials and methods**: Study at Biochemistry lab of Hue University of Medicine and Pharmacy, we have performed 30 patients with stage 3-4 CKD and 30 controls. **Results**: CKD patients presented the prevalence of hypertension was significantly higher in CKD patients than controls (66,7%; 0%, respectively, p<0.001); plasma levels of malondialdehyde were progressively lower in CKD patients (median=4.23 µmol/L, range=1.37 - 11.01) than controls (median=5.04 µmol/L, range=1.01 - 8.18) but there was no important difference between 2 groups; CKD patients present higher plasma levels of kynurenine, consequently, higher kyn/trp ratio (median=0.054; IQR 0.044 - 0,095 vs 0.030; IQR 0.020 - 0.040, p<0.001) compared to healthy controls and the increase of kyn/trp ratio was progressively higher with CKD late stage; kyn/trp ratio as a biomarker has predictive ability to discriminate CKD from normal subjects (AUC: 0.87; 95% CI: 0.78-0.96; p<0.001); there was a correlation between Kyn/Trp ratio and eGFR. **Conclusions**: In addition to the significant alteration in the Kyn/Trp ratio, we also found that there was a correlation between Kyn/Trp ratio and eGFR. About malondialdehyde, required confirmation of our results in larger study cohorts to fully featured the impact of oxidative stress in this pathology.

Keywords: *CKD, Chronic kidney disease, kynurenin, malondialdehyde, inflammation, oxidative stress, tryptophan.*

1. BACKGROUND

Chronic kidney disease (CKD) is recognized as a major global health problem, go along with a number of serious complications. There are several risk factors in CKD patients that could be separated into traditional and nontraditional risk factors. Diabetes mellitus, older age, hypertension, and hyperlipidemia are traditional risk factors commonly present in the CKD population [1]. Oxidative stress and inflammation are considered nontraditional risk factors. The imbalance between reactive oxygen species (ROS) production and antioxidant defenses induces oxidative stress. This state is predominant in CKD and also accelerates renal injury progression [2]. Lipid peroxidation products such as malondialdehyde (MDA) have been used as biomarkers of oxidative stress by the elevation of MDA in CKD [3]. In addition, inflammation facilitates renal function deterioration. Several factors can be involved in triggering the inflammatory process including oxidative stress. Tryptophan (Trp) is a fundamental amino acid for humans, and its metabolism produces various bioactive substances involved in the pathophysiology of CKD. The Kyn-to-Trp ratio has been proposed as a sensitive tool for evaluating inflammation status. Kynurenine (Kyn) is a metabolite of Trp through kynurenine pathway, and the expression of metabolic enzyme can be induced by proinflammatory cytokines, which is upregulated in earlier response to tissue inflammation [4], [5].

In this context, we aimed to evaluate the plasma biomarker indexes of oxidative stress and inflammation in CKD patients to assess its value in the surveillance of CKD.

Research objectives: *1. To evaluate the plasma MDA concentration and Kyn/Trp ratio for differences between CKD patients and healthy controls; 2. To analyze the relationship and correlation between plasma MDA concentration and Kyn/Trp ratio and some risk factors of CKD.*

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2. MATERIALS AND METHODS

2.1. Materials

We conducted plasma samples and recorded data from 30 chronic kidney disease patients in stages 3-4 and 30 control samples from April 2019 to August 2019. We recorded personal information, blood pressure, plasma creatinine, urea results.

Chemicals: MDA standard, SDS, TRIS-HCl, Thiobarbituric acid, Kyn and Trp stock standard, Methyl tryp (internal standard), Acetonitrile, PBS, Bis tris propane buffer (Sigma-Aldrich), HCl, NaOH, acid acetic (Merck).

Instrumentation-equipment: Capillary electrophoresis equipped with a UV detector (Beckman Instruments, Brea CA, USA), UV-Vis Spectrophotometer with cuvettes, Sigma-1-14-microfuge, Rotina 420 centrifuge (Hettich Germany), Digital pH meter 3 points calibration (HANNA instrument), Voxtex mixer, Thermo scientific digital dry baths incubation/Block heater (Fisher scientific), Freezer, -80°C -20°C, 4°C, Micropipettes: p10, p20, p100, p1000 (Socorex, Switzerland).

2.2. Methods

2.2.1. MDA and Kyn, Trp measurement

MDA levels were measured according to the spectrophotometric measurement of the color that occurred during the reaction of thiobarbituric acid with MDA.

Tryptophan and kynurenine quantification were determined by capillary electrophoresis equipped with a UV detector, as described in Zinellu 2012 [6].

2.2.2. Method validation

Statistical analyses were performed using SPSS for Windows, version 20.0 64 bit (IBM Corporation, NY, USA) and Microsoft excel 2013.

3. RESULTS

The study was conducted on 60 subjects and divided into two main groups: the healthy controls and stage 3-4 chronic kidney disease patients.

3.1. Characteristics of study subjects

Table 1. Clinical and functional parameters of healthy subjects and CKD patients

Characteristics	Control (n=30)	CKD (n=30)	P value*
Age (years)	62.5 (59 - 70)	68.5 (57 - 78.8)	0.297
Gender, n (%)			
Male	13 (43.3)	18 (60.0)	0.196
Female	17 (56.7)	12 (40.0)	
BMI (kg/m^2)	21.5 (20.82 - 24.56)	23.1 (20.8 - 24.3)	0.367
Smoking, n (%)			
No	18 (60.0)	18 (60.0)	1.000
Yes	12 (40.0)	12 (40.0)	
Systolis (mmHg)	120 (120 - 130)	140 (130 - 150)	<0.001
Diastolic (mmHg)	70 (70 - 80)	80 (80 - 90)	<0.001
Hypertension, n (%)			
No	30 (100)	10 (33.3)	<0.001
Yes	0 (0)	20 (66.7)	

Table 2. Renal function parameters of healthy subjects and CKD patients

	Control (n=30)	CKD (n=30)			p_1	p_2
		CKD stage 3 (eGFR 30-60)	CKD stage 4 (eGFR 15-30)	Total		
Ure (mmol/L)	4.8 (4.1-5.4)	9.3 (7.3-11.4)	12.7 (9.3-15.6)	10.3 (8.5-13.5)	0.018	<0.001
Creatinine (µmol/L)	66.5 (59-74)	152 (128-173)	236 (184-270)	187.5 (162-251)	<0.001	<0.001
eGFR (mL/min/1.73m^2)	91.7 (85.6-101.5)	36.8 (31.3-43.2)	24.1 (19.8-26.8)	26.8 (23.7-34.3)	<0.001	<0.001

3.2. Plasma malondialdehyde concentration; Tryptophan and kynurenine results

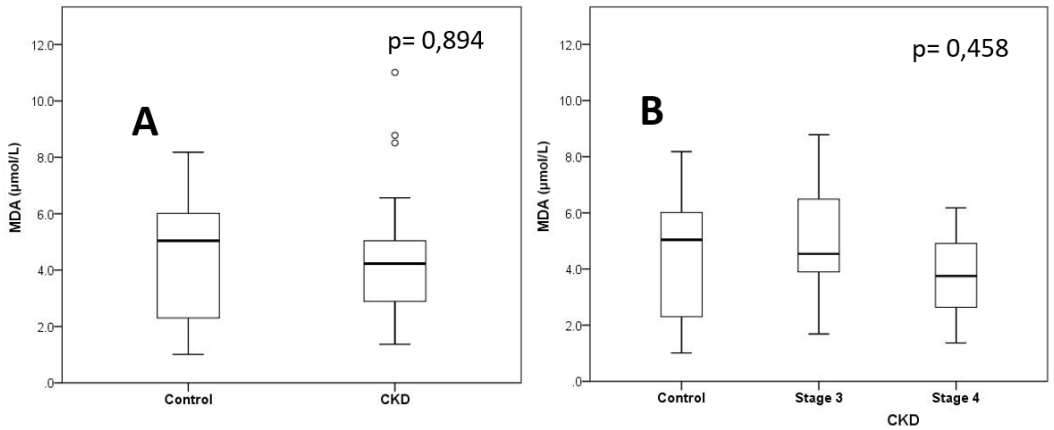

Figure 1. In part A are shown plasma levels of MDA, in healthy subjects and CKD patients; in part B are shown plasma levels of MDA in healthy subjects and CKD patients after sorting for disease stages.

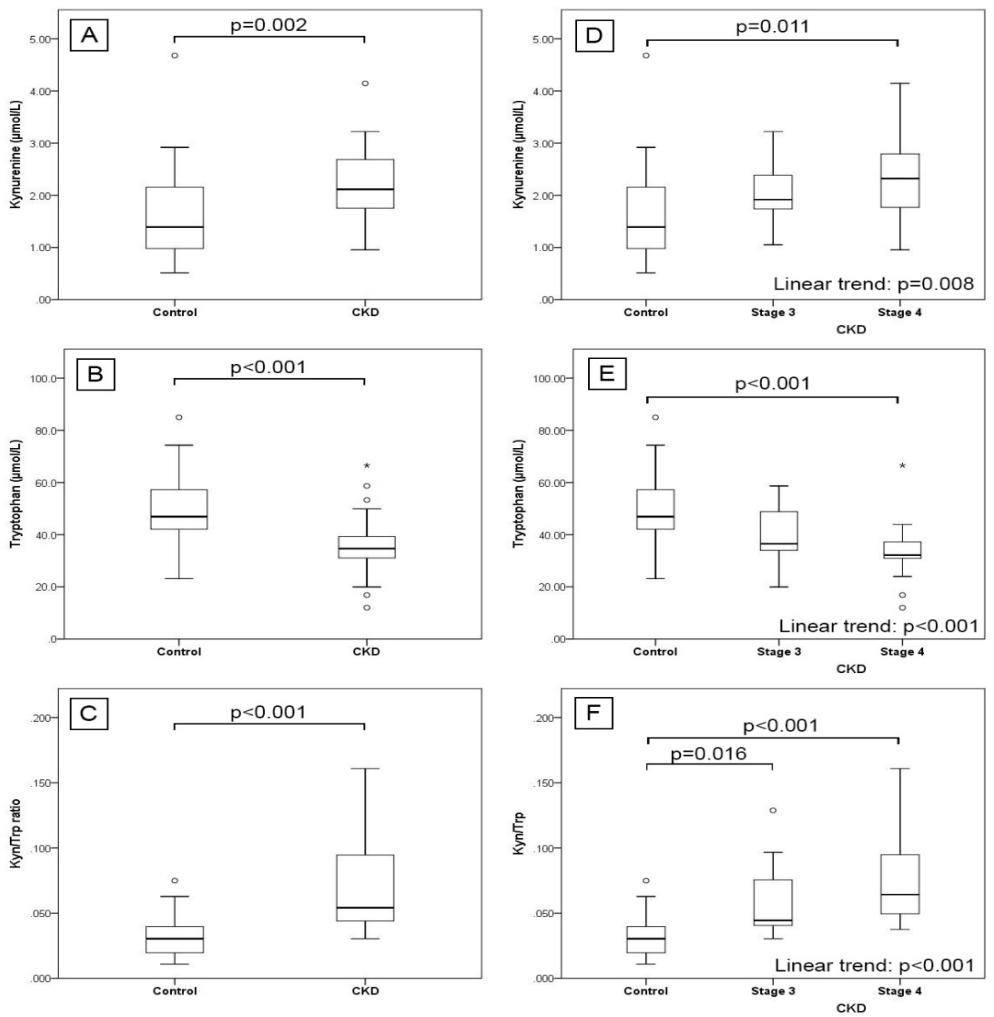

Figure 2. In part A, B and C are shown plasma levels of kynurenine, tryptophan and Kyn/Trp ratio in healthy subjects and CKD patients; in part D, E and F are shown plasma levels of kynurenine, tryptophan and Kyn/Trp ratio in healthy subjects and CKD patients after sorting for disease stages.

Table 3. Prognostic accuracy of the kynurenine/tryptophan ratio alone or in combination with hypertension

Marker	AUC	95% CI	p	Cut-off	Sens.	Spec.
Kyn/Trp	0.87	0.78 - 0.96	<0.001	>0.04	86.7	76.7
Kyn/Trp+ Hypertension	0.93	0.87 - 0.99	<0.001	>0.646	80.0	96.7

AUC: Area under the curve; CI: Confidence interval
Kyn/Trp+Hypertension: Combination of Kyn/Trp and Hypertension
Sens. : Sensitivity Spec. : Specificity

3.3. The relation and correlation MDA, Trp, Kyn, Trp/Kyn ratio and some risk factors

Table 4. Relation between oxidative stress/inflammation markers and age

Age	CKD patients		P*
	< 65 years (n=13)	**≥ 65 years (n=17)**	
	Median (Q1 - Q3)	Median (Q1 - Q3)	
MDA	4.84 (2.48 - 6.06)	3.84 (2.77 - 4.97)	0.869
Kyn	2.32 (1.78 - 2.99)	1.92 (1.66 - 2.48)	0.229
Trp	33.51 (31.04 - 43.55)	35.08 (28.05 - 39.97)	0.869
Kyn/Trp	0.056 (0.044 - 0.1)	0.051 (0.043 - 0.087)	0.592

Table 5. Relation between oxidative stress/inflammation markers and hypertension

	CKD patients		P*
	Non hypertension (n = 10)	**Hypertension (n = 20)**	
	Median (Q1 - Q3)	Median (Q1 - Q3)	
MDA	4.47 (1.98 - 5.39)	4.11 (3.24 - 5.71)	0.746
Kyn	2.11 (1.71 - 2.92)	2.12 (1.77 - 2.64)	0.983
Trp	35 (31.84 - 48.32)	34.62 (26.69 - 37.95)	0.350
Kyn/Trp	0.048 (0.039 - 0.09)	0.06 (0.045 - 0.096)	0.267

Table 6. Correlation between plasma levels of MDA, kynurenine, tryptophan and Kyn/Trp ratio and systolic BP, eGFR in CKD

Variables		Systolic BP	eGFR
MDA	r	- 0.043	0.058
	p	0.742	0.658
Kynurenine	r	0.319	- 0.364
	p	0.013*	0.004
Tryptophan	r	- 0.438	0.618
	p	< 0.001	<0.001
Kyn/Trp	r	0.536	- 0.629
	p	< 0.001	<0.001

4. DISCUSSION

4.1. Characteristics of study subjects

CKD patients and healthy controls were individually matched on some characteristics: age, gender, weight, height, BMI and smoking status. 30 CKD patients with median age=69, min=45, max=86 years and 60% male are similar with Zinellu et al (age 60.2 ± 10.5 years, 63% male) [4]. In these

aging-related disorders, characterized by increases of oxidative stress-malondialdehyde play important pathogenic roles [7]. Moreover, a chronic pro-inflammatory status is a pervasive feature of aging. Recently, several possible sources of chronic low-grade inflammation observed during aging and age-related diseases have been proposed [8]. Biomarkers of inflammation, oxidative stress, immunity, tissue injury and repair were elevated in cigarette smokers or obesity [9], [10]. Therefore, it is important to be no difference about these statuses between two groups.

CKD patients presented high systolis, diastolic BP and the prevalence of hypertension was significantly higher in CKD patients than controls (66.7%; 0%, respectively, p<0.001). CKD and hypertension are closely associated with an overlapping and intermingled cause and effect relationship. Declines in kidney function are typically associated with rises in BP, and sustained elevations in BP hasten the progression of kidney function decline. In the Chronic Renal Insufficiency Cohort, which consists of 3612 adults with CKD (majority moderate stage), the prevalence of self-reported hypertension was 86% compared with 29% in the general population. Furthermore, the prevalence rate of hypertension rises, and BP becomes more difficult to control with advancing CKD stage [11].

CKD patients presented a decrease of renal functionality (higher median of plasma urea, creatinine and lower median of eGFR) respect of healthy controls (p<0.001). eGFR decrease is further greater with the progression of the disease (stage 4 kidney disease got lower eGFR median than those in stage 3, p<0.001). It is clear to confirm the significant difference because it depends on the diagnosis and classification of the stage of CKD.

4.2. Plasma malondialdehyde concentration; Tryptophan and kynurenine results

In our results, there was no important difference between two groups about plasma levels of malondialdehyde (p>0.05). We expected the result that MDA concentration will be higher in CKD patients. The biggest limitation of this study deserve mention was that the number of CKD samples are still small, not enough quantity so that it affects the accuracy of statistics. Gaosi Xu el al confirmed with the development of CKD, serum levels of MDA were significantly increased in these participants [12]. One more study highlighted the importance of evaluating the three forms of MDA in order to understand the role of oxidative stress, especially in patients with reduced renal function [13]. Therefore, it might be interesting to increase the number of subjects of the study to fully characterized the impact of oxidative stress in this pathology.

In this report, it showed that CKD patients are characterized by higher plasma concentrations of kynurenine and lower concentrations of tryptophan. Furthermore, in stage 4 CKD patients, with the progression of the disease stage, kynurenine concentrations further increased, while tryptophan concentrations significantly decreased and, as a consequence, also the kyn/trp ratio increased (median=0.054 µmol/L; IQR 0.044-0.095 µmol/L vs 0.030 µmol/L; IQR 0.020-0.0400 µmol/L, p<0.001) (figure 2). In accordance with a recent observation we found higher levels of plasma kynurenyne and that recurrent or chronic inflammatory processes are common in CKD [14]. Another study indicated the similar results, namely, baseline Kyn and Kyn/Trp ratio were higher in CKD patients vs. healthy controls (1.67 ± 0.62 µmol/L vs 1.25 ± 0.40 µmol/L, p<0.01 and 0.036 ± 0.016 vs 0.023 ± 0.010, p<0.001 respectively) [4]. These results suggest the possible increase of IDO activity, due to the inflammatory processes common in CKD [5]. It is completely explanation with these results.

The ROC analysis showed the significant ability of kyn/trp ratio to discriminate CKD patients from controls with 86.7% sensitivity and 76.7%. The Kyn/Trp ratio in combination with hypertension and results obtained considerably improved, with an AUC of 0.93, sensitivity of 80% and specificity of 96.7%. They show that kyn/trp ratio as a biomarker has predictive ability to discriminate chronic kidney disease from normal subjects. Zhao J. et al concluded that plasma Kyn acid/Trp ratio is sensitive and reliable to indicate renal function and could be utilized as a new biomarker for the diagnosis of kidney disease, as well as its severity [14]. Benitez T. et al demonstrated a possible role for altered tryptophan immune metabolism (low Trp, high Kyn) in the pathogenesis of CKD-associated atherosclerosis [15].

4.3. The relation and correlation plasma MDA, Trp, Kyn, Trp/Kyn ratio and some risk factors

Our data showed clearly no independently significant relations between oxidative stress and inflammation indices and groups of age, smoking status and hypertension in CKD group (p>0.05). They are risk factors in CKD that demonstrated via previous study. However, in our study, we did not find the relations. The important reason could be the sample size to affect the results.

As expected, there was the correlation between

plasma kynurenine, tryptophan, Kyn/Trp ratio and Systolic BP, eGFR in CKD patients. Between CKD and hypertension has a strong relation that demonstrated via previous study. BP typically rises with declines in kidney function, and sustained elevations in blood pressure hasten progression of kidney disease [16]. Pecoits Filho R. et al showed a strong association between low GFR and an increased proinflammatory activity in patients with advanced renal impairment. They also provide indirect evidence that this association is not influenced entirely by the presence of comorbidities, but may represent the impact directly or indirectly of decreased kidney function per second in the development of the inflammatory milieu [17]. Furthermore, Gaosi Xu et al showed inflammation and oxidative stress interacted with each other and played pivotal roles in the development of CKD. Variation in eGFR was parallel with the changes of oxidative stress and inflammation when CKD developing [12]. On the other hand, Payson Oberg B. Et al indicated that there was no significant relationship between estimated GFR and any oxidative stress or inflammation biomarker [18]. The relatively small number of participants requires confirmation of our results in larger study cohorts.

In light of the apparently strong impact of inflammation in chronic kidney disease patients, further studies are needed to address the role of the kidney in the clearance and inactivation of inflammatory mediators. About oxidative stress, the relatively small number of participants required confirmation of our results in larger study cohorts and to include patients with late stage of CKD to fully featured the impact of oxidative stress in this pathology.

5. CONCLUSIONS

Summary these data clearly confirm that CKD patients have presented high levels of kynurenine and Kyn/Trp plasma ratio, low levels of tryptophan compared to healthy controls. In addition, the alterations of these biomarkers are further greater with the stage of disease. It also shows for the first time that Kyn/Trp ratio as a biomarker has predictive ability to discriminate chronic kidney disease from normal subjects. We also found that there was a correlation between kynurenine, tryptophan, Kyn/Trp ratio and eGFR. However, our data indicate no statistically significant difference about plasma malondialdehyde concentration in chronic kidney disease from healthy controls. These were just the initial result. Therefore, this status requires further larger, appropriately powered studies and to include patients with late stage of CKD to fully characterized the impact of oxidative stress and inflammation in this pathology.

REFERENCES

1. Kao MP., Ang DS., Pall A., & Struthers AD. Oxidative stress in renal dysfunction: mechanisms, clinical sequelae and therapeutic options. Journal of Human Hypertension. 2010 Sep; 24(1): 1–8.

2. Modaresi A., Nafar M., & Sahraei Z. Oxidative stress in chronic kidney disease. Iranian Journal of Kidney Diseases. 2015 May; 9(3): 165–179.

3. Sung C. C., Hsu Y. C., Chen C. C., Lin Y. F., & Wu C. C. Oxidative stress and nucleic acid oxidation in patients with chronic kidney disease. Oxidative medicine and cellular longevity. 2013 Aug; 2013:1-15.

4. Zinellu A., Sotgia S., Mangoni A., Sanna M., Satta AE., & Carru C. Impact of cholesterol lowering treatment on plasma kynurenine and tryptophan concentrations in chronic kidney disease: Relationship with oxidative stress improvement. Nutrition, Metabolism and Cardiovascular Diseases. 2015 Feb; 25(2): 153–159.

5. Zakrocka, Izabela; ZAŁUSKA, Wojciech. Kynurenine pathway in kidney diseases. Pharmacological Reports. 2021 Oct; 74(1): 27-39.

6. Zinellu A., Sotgia S., Deiana L., Talanas G., Terrosu P., & Carru C. Simultaneous analysis of kynurenine and tryptophan in human plasma by capillary electrophoresis with UV detection. 2012 Jun; 1146–1151.

7. Barrera G., Pizzimenti S., Daga M., Dianzani C., Arcaro A., Cetrangolo GP., et al. Lipid peroxidation-derived aldehydes, 4-hydroxynonenal and malondialdehyde in aging-related disorders. Antioxidants. 2018 Jul; 7(8): 102.

8. Dugué P. A., Hodge A. M., Ulvik A., Ueland P. M., Rinaldi S., Giles G. G., et al. Association of markers of inflammation, the kynurenine pathway and B vitamins with age and mortality, and a signature of inflammaging. The Journals of Gerontology: Series A. 2022 Apr; 77(4): 826-836.

9. Iglesias P., & Díez JJ. Adipose tissue in renal disease: clinical significance and prognostic implications. 2010 May; 2066–2077.

10. Khan NA., Lawyer G., McDonough S., Wang Q., Kassem NO., Rahman I., et al. Systemic biomarkers of inflammation, oxidative stress and tissue injury and repair among waterpipe, cigarette and dual tobacco smokers. Tobacco Control. 2019 Sep; 29(Suppl 2): 102 - 109.

11. Lash J. P., Go A. S., Appel L. J., He J., Ojo A., Rahman M., et al. Chronic Renal Insufficiency Cohort (CRIC) Study: baseline characteristics and associations with kidney function. Clinical Journal of the American Society of Nephrology. 2009 Aug; 4(8): 1302-1311.

12. Xu G., Luo K., Liu H., Huang T., Fang X., & Tu W. The progress of inflammation and oxidative stress in patients with chronic kidney disease. Renal Failure. 2014 Nov; 37(1): 45–49.

13. Mas-Bargues C., Escriva C., Dromant M., Borras C., & Vina J. Lipid peroxidation as measured by chromatographic

determination of malondialdehyde. Human plasma reference values in health and disease. Archives of biochemistry and biophysics. 2021 Jun; 709: 108941.
14. Zhao J. Plasma kynurenic acid/tryptophan ratio: A sensitive and reliable biomarker for the assessment of renal function. Renal Failure. 2013 May; 35(5): 648–653.
15. Benitez T., VanDerWoude E., Han Y., Byun J., Konje V. C., Mathew A. V., et al. Kynurenine pathway metabolites predict subclinical atherosclerotic disease and new cardiovascular events in chronic kidney disease. Clinical Kidney Journal. 2022 Oct; 15(10): 1952-1965.
16. Bidani AK., Polichnowski AJ., Loutzenhiser R., & Griffin KA. Renal microvascular dysfunction, hypertension and CKD progression. Current Opinion in Nephrology and Hypertension. 2013 Jan; 22(1): 1–9.
17. Pecoits-Filho R., Heimbürger O., Bárány P., Suliman M., Fehrman-Ekholm I., Lindholm B., et al. Associations between circulating inflammatory markers and residual renal function in CRF patients. American Journal of Kidney Diseases. 2003 Feb; 41(6): 1212–1218.
18. Oberg BP., McMenamin E., Lucas E., McMonagle E., Morrow J., Ikizler P., et al. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney International. 2004 Mar; 65(3): 1009–1016.