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Anthranilic acid as an uremic toxin leading to alteration of the fibrinolytic system in pre-dialysis patients with chronic kidney disease

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Introduction and objectives

Chronic kidney disease (CKD) is linked to increased retention of a number of the uremic toxins forming an uremic *milieu*. Previous studies reported CKD environment as highly thrombogenic as well as increasing risk of bleeding in CKD patients [1,2]. Anthranilic acid (AA) is derived from tryptophan (Trp) uremic toxin exerting proinflammatory and prooxidative properties, which may affect proper maintenance of hemostatic balance [3]. The objective of the study was to examine the association between AA concentrations and the alteration of the fibrinolytic system, oxidative status, inflammation, and immunological response in pre-dialysis patients with CKD and then compare obtained results to healthy individuals.

Methods

Studies have been conducted on a group of 48 pre-dialysis patients with CKD on conservative treatment and 18 healthy volunteers. The overall CKD group was divided into 2 subgroups in view of CKD progression: subgroup B including mild to moderate CKD (II and III stages of CKD, n=20), and subgroup C – severe to end-stage CKD (IV and V stages of CKD, n=28). ELISA-immuno-enzymatic kits were used for the determination of parameters of fibrinolysis, whereas the AA, Trp, and kynurenine (Kyn) levels were determined by high performance liquid chromatography. The markers of inflammation, oxidative stress, and immunology status were also assayed using ELISA kits. The hematological and biochemical parameters were assessed using standard laboratory methods. The comprehensive computations were performed using GraphPad Prism 6 software.

Results and Conclusions

Parameter	Controls n=17	CKD n=48	Mild to Moderate CKD n=20	Severe to End-stage CKD n=28
Sex M/F	7/10	19/29	8/12	11/17
Age, years	47.7 ± 6.23	52.9 ± 15.7	50.5 ± 18	54.7 ± 15.9
BMI, kg/m^2	26.1 ± 3.37	23.9 ± 3.34	24.4 ± 3.11	23.6 ± 3.61
eGFR, mL/min/1.73m^2	117 (105 – 125)	19.9 (5.6 – 127)***	58.8 (29.8 – 127)* ^^^	12.4 (5.6 – 28.9)*** ^^ ###
Creatinine, mg/dL	0.89 (0.29 – 1.18)	3.45 (0.78 – 7.83)***	1.2 (0.78 – 2.31)** ^^^	5.03 (1.82 – 7.83)*** ^^ ##
Urea, mg/dL	30 ± 6.23	114 ± 49.5***	88.9 ± 54.1***	137 ± 42*** ^ ##
hs-CRP, µg/ml	1.15 (0.01 – 10.9)	2.98 (0.01 – 47)*	2.03 (0.01 – 45)	5.85 (0.02 – 47)**
Neopterin, nmol/L	6.18 (0.4 – 12.91)	31.4 (5.1 – 110)***	28 (5 – 108)***	39.1 (5 – 110)*** #
H_2O_2, µM	52.4 (2.33 – 408)	243 (60.7 – 423)***	201 (67.8 - 379)**	275 (60.7 – 423)***
Glucose, mg/dL	88 (67 – 114)	90 (45 – 155)	95.5 (76 – 155)	89 (45 – 121)#
Cholesterol mg/dL	193 (144 – 245)	198 (106 – 485)	194 (164 – 485)	200 (106 – 263)
Triglycerides, mg/dL	67 (38 – 149)	148 (61 – 481)***	170 (61 – 620)***	147 (63 – 392)***
Total protein, g/dL	6.41 ± 0.27	6.01 ± 0.94	5.78 ± 0.89**	6.21 ± 0.99
Albumin, g/dL	4.41 ± 0.17	3.3 ± 1.18***	3.77 ± 1.13**	3.18 ± 1.27*** #
Bilirubin, mg/dL	0.34 ± 0.13	0.45 ± 0.2	0.43 ± 0.21	0.51 ± 0.21**
ALT, U/L	33 (16 – 53)	27.5 (10 – 120)	28 (15 – 120)	25.5 (10 – 58)
Red blood cells,10^3 µL	4.55 ± 0.32	3.55 ± 0.68***	3.97 ± 0.77** ^	3.31 ± 0.54*** ###
White blood cells,10^3 µL	5.76 ± 1.13	6.38 ± 2	6.93 ± 1.99*	5.99 ± 1.95
Lymphocytes, %	33.7 ± 4.89	26.5 ± 8.98**	30.9 ± 9.52^	24.1 ± 8.07*** ##
Platelets, x10^3 µL	206 (132 – 312)	183 (76 – 376)	219 (115 – 359)	175 (76 – 376)
Neutrophils, %	58.3 ± 5.47	58.8 ± 12.4	57.5 ± 13.1	60.6 ± 12.8
Hemoglobin, g/dL	14.2 ± 1.32	11 ± 2.21***	12.2 ± 2.52* ^	10.3 ± 1.82*** ##
Hematocrit, %	42.2 ± 3.2	32.9 ± 5.99***	36.2 ± 6.81** ^	31 ± 4.96*** ##

Table I. Biochemical and clinical characteristics of control group and uremic patients.
Legend: */**/*** - *P* values respectively <0.05; <0.01; <0.001 (Controls vs. others)
^/^^/^^^ - *P* values <0.05; <0.01; <0.001 (CKD vs. others)
#/##/### *P* values <0.05; <0.01; <0.001 (group B vs. group C)

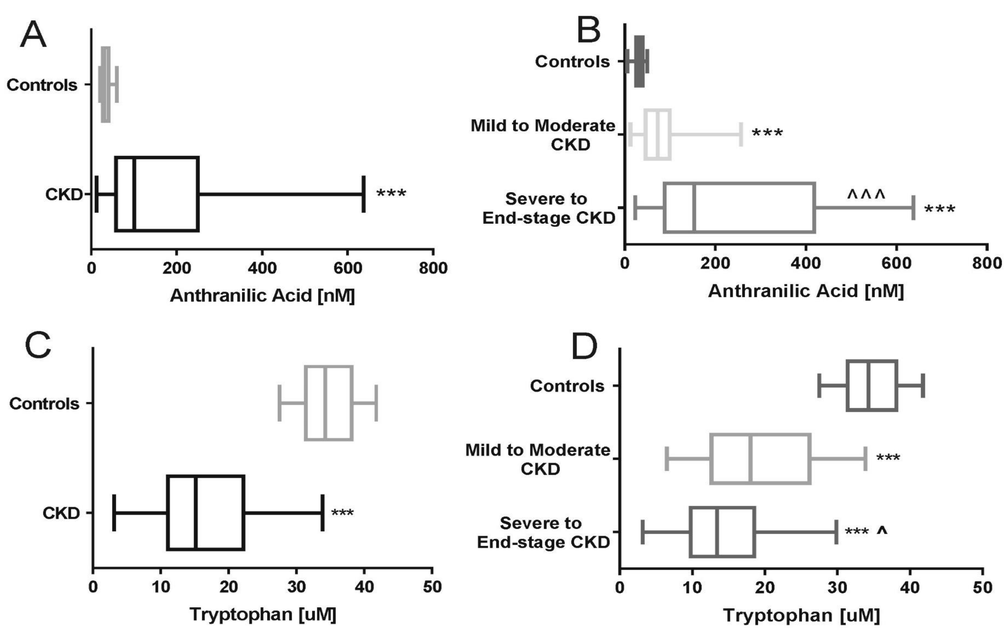

Figure 1: The levels of AA and Trp in control group and CKD group (A,C) and comparison of the levels of AA and Trp among the patients with the different stage of CKD (B,D).
Legend: */**/*** - P values respectively <0.05; <0.01; <0.001 (Controls vs. others).
^/^^/^^^ - P values <0.05; <0.01; <0.001 (Severe to End-stage CKD vs. Mild to Moderate CKD)

Factor	Controls	Overall CKD	Mild to Moderate CKD Group (B)	Severe to End-stage CKD Group (C)
tPA [ng/ml]	5.1 (2.8 – 9)	**6.51 (2.4 – 53.6)***	**6.5 (3.6 – 17.3)****	6.8 (2.7 – 53.6)
uPA [ng/ml]	0.4 (0,1 – 1.1)	**1.2 (0.6 – 5)*****	**1.1 (0.7 – 2.15)*****	**1.28 (0.6 – 5)*** #**
suPAR [ng/ml]	0.09 (0.07 – 0.2)	**2.31 (0.6 – 5.78)*****	**1.38 (0.6 – 5.7)*** ^**	**2.67 (1.3 – 4.8)***^##**
PAI-1 [ng/ml]	24.5 (13 – 79)	**55.5 (12 – 86.8)*****	**56.5 (30.7 – 77.8)*****	**54.8 (13.9 – 86.8)****
PAP [ng/ml]	211 (31 – 457)	**466 (84.2– 1611)****	**445 (104 – 1611)***	**470 (103 – 1525)***

Table II. Parameters of the fibrinolytic system in healthy controls, overall CKD patients, and comparison of the fibrinolysis factors in the CKD subgroups.
Legend: */**/*** - *P* values respectively <0.05; <0.01; <0.001 (Controls vs. others).
^/^^^ - *P* values <0.05 and <0.001 (CKD vs. others)
#/### *P* values <0.05 and <0.001 (group B vs. group C)
Abbreviations**: tPA – tissue plasminogen activator, uPA – urokinase-type plasminogen activator, suPAR – soluble urokinase-type plasminogen activator receptor, PAI-1 – plasminogen activator inhibitor-1, PAP – plasmin-α2-antiplasmin.**

Overall CKD	R	*P* value
tPA	**-0.365**	**0.0115**
uPA	**0.345**	**0.0176**
suPAR	**0.470**	**0.0009**
PAI-1	**-0.393**	**0.0062**
PAP	0.0429	NS
Mild to Moderate CKD	R	*P* value
tPA	0.103	NS
uPA	**0.689**	**0.0008**
suPAR	**0.561**	**0.0125**
PAI-1	-0.209	NS
PAP	0.152	NS
Severe to End-stage CKD	R	*P* value
tPA	**-0.573**	**0.0018**
uPA	-0.023	NS
suPAR	0.029	NS
PAI-1	**-0.458**	**0.0188**
PAP	0.0194	NS

Table III. Correlations between the levels of AA and the parameters of fibrinolytic system in overall CKD, mild to moderate CKD subgroup, and severe to end stage CKD subgroup. Results are shown as Spearman's rank correlations coefficient (R) and its statistical significance (*P* value).

Overall CKD	Independent variable	Regression coefficient	P value
tPA	AA	-0.28	0.034
	uPA	0.32	0.036

Table IV. Variables independently associated with tPA levels in overall CKD group. Multiple R for variables in the model – 0.682, multiple R^2 – 0.466, adjusted R^2 – 0.388, p<0.0015. Included variables: AA, Trp, Kyn, fibrinolytic factors, renal insufficiency markers.

Our research has shown for the first time association between the level of AA and the parameters of the fibrinolytic system in a group of CKD patients on conservative treatment. The distinct relationship between AA and individual parameters of fibrinolytic system seem to be dependent on the stage of CKD. The obtained data suggest the existence of previously unknown properties of AA towards the fibrinolytic disturbances in this population. Although at this stage our results are only speculative, we can assume that AA may be the part of various mechanisms involved in the alteration of the fibrinolytic system during CKD. Additional experimental support is needed to resolve several crucial issues in order to fully understand observed phenomena.

Identifier/Topic: CDK - NUTRITION & INFLAMMATION I :

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