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Accumulation of kynurenine pathway metabolites in saliva and plasma of uremic patients

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Abstract:
A marked increase in toxic metabolites of the L-tryptophan: kynurenine pathway, kynurenine (KYN) and quinolinic acid has been observed in serum and cerebrospinal fluid of both, rats and humans with renal insufficiency. Recently, we have found increased concentration of KYN and kynurenic acid (KYNA), but not 3-hydroxykynurenine (3-HKYN) and anthranilic acid (AA) in saliva of hypertensive diabetic patients.
The aim of the study was to estimate certain kynurenine derivatives in plasma and saliva of uremic patients.
The concentration of KYN and its metabolites were estimated in 19 uremic patients and 19 healthy volunteers by high-performance liquid chromatography (HPLC).
The increase in the concentration of KYN, 3-HKYN, KYNA and AA was observed in plasma of uremic patients in comparison with the control group (3.1 ± 1.2 μM, 415.9 ± 120.8 nM, 339.4 ± 189.0 nM and 611.7 ± 274.7 nM *vs*. 1.6 ± 0.5 μM, 35.6 ± 10.0 nM, 28.0 ± 7.3 nM and 35.1 ± 4.6 nM; $p < 0.0001$, $p < 0.0001$, $p < 0.0001$ and $p < 0.0001$, respectively). Also in saliva the concentration of KYN (35.1 ± 24.0 nM; $p < 0.0001$), 3-HKYN (24.5 ± 18.2 nM; $p < 0.0001$), KYNA (108.6 ± 72.4 nM; $p < 0.0001$) and AA (111.6 ± 46.4 nM; $p < 0.0001$) was increased in uremic patients in comparison with the values observed in healthy volunteers (25.8 ± 10.8 nM, 1.1 ± 0.5 nM, 5.7 ± 3.3 nM and 13.7 ± 6.2 nM, respectively).
The increased concentration of KYN, 3-HKYN, KYNA and AA in plasma and saliva of uremic patients in comparison with healthy volunteers suggests an altered metabolism of kynurenine in uremia.

Key words:
kynurenine pathway metabolites, uremia, saliva

Abbreviations: AA – anthranilic acid, con – control group, 3-HKYN – 3-hydroxykynurenine, HPLC – high performance liquid chromatography, KYN – kynurenine, KYNA – kynurenic acid, pre-HD – prehemodialysis, TDO – tryptophan 2,3-dioxygenase

Introduction

In uremia, several endogenous metabolites accumulate in the blood, including products of tryptophan (TRP) degradation via kynurenine pathway [16]. Accumulation of these metabolites in uremic blood is through to contribute to certain uremic symptoms, such as neurological disturbances [22], hypertension [15], lipid metabolism disorders [14] and anemia [17]. The family of kynurenine (KYN) metabolites includes compounds that have been identified as neurotransmitter agonists [18] and antagonists [9, 10, 21], neurotoxins [12], immunomodulators and antioxidants [19].

Uremic stomatitis is characterized by the presence of painful plaques and crusts that are usually distributed on the buccal mucosa, dorsal or ventral surface

of the tongue, gingiva, lips and floor of the month, and it is an oral finding in subjects with chronic renal failure [1]. Pathogenesis of stomatitis in uremia is not yet definitely established. It may develop due to intrinsic and/or extrinsic factors. Recently, Saito et al. [16] have demonstrated a marked increase in toxic metabolites of the kynurenine pathway, KYN and quinolinic acid, in serum and cerebrospinal fluid of rats and humans with renal insufficiency.

In the current study, we decided to investigate plasma and saliva KYN, 3-hydroxkynurenine (3-HKYN), kynurenic acid (KYNA) and anthranilic acid (AA) concentrations in uremic patients. The evaluation of KYN and its metabolites was performed in order to study the pathophysiology of uremic stomatitis lesions.

Materials and Methods

Subjects

The study was performed on 19 clinically stable hemodialyzed patients (10 males and 9 females), aged 33–79 years (mean age 63 ± 16). These patients met the following criteria: absence of cardiovascular complications (including uncontrolled hypertension), no oral contraception in women of child-bearing age, no evidence of blood loss during the last 6 months other than that related to dialysis, and no other than renal cause of anemia. None of the enrolled patients had received blood transfusion for at least 3 months, and no drugs affecting TRP metabolism were administrated 2 weeks before blood and saliva collection. The causes of renal failure among hemodialyzed patients varied between chronic glomerulonephritis (n = 5), chronic interstitial nephritis (n = 5), diabetic nephropathy (n = 5) and other or unknown causes (n = 4).

The control group consisted of 19 healthy volunteers (9 males and 10 females), aged 29–76 years (mean age 61 ± 15).

In every patient, blood was collected in the morning between 8:00 and 9:00 a.m. before the onset of dialysis session and heparin administration. In the control group blood was obtained in the morning between 8:00 and 9:00 a.m.

The study was approved by the local Ethics Committee (R-I-003/106/2006). All participants provided written informed consent.

Blood sampling

Blood was collected from the a-v fistula before the onset of dialysis session into a tube containing of 3.8% sodium citrate (citrate/blood = 1/9). Platelet poor plasma was obtained by blood centrifugation at 3000 rpm for 15 min (temp. 4°C). Samples were stored at –80°C until assayed.

Saliva sampling

Samples of non-stimulated, mixed saliva were taken from studied subjects each morning between 7:00 and 8:00 a.m., 10 min after mouth washing with MilliQ water. The saliva samples were immediately treated 2 M $HClO_4$. After 15 min of incubation with the acid at 4°C, samples were centrifuged 12000 × g for 30 min and the supernatant was collected and stored at –80°C.

Determination of kynurenine and its metabolites

In order to estimate KYN, 3-HKYN, KYNA and AA concentrations, the plasma and saliva were immediately deproteinized with 2 M $HClO_4$ and centrifuged at 12,000 × g for 15 min at 4°C. The supernatant fluid was passed through a WATERS 0.45 μM filters. Samples were stored at –80°C until assayed. The studied metabolites were determined by high-performance liquid chromatography (HPLC): KYN with UV detection [8], 3-HKYN with electrochemical detection [6], KYNA and AA with fluorescence detection [5].

Statistical analysis

The values are expressed as the mean ± SD; n – represents the number of results. Statistical analysis was done using Student's *t*-test; p value less than 0.05 was considered statistically significant.

Results

Baseline characteristics of patients included in the study are presented in Table 1. Figures 1 and 2 present the concentrations of KYN, 3-HKYN, KYNA and AA in plasma and saliva of patients with chronic renal failure, respectively.

In uremic patients, plasma concentrations of KYN ($p < 0.0001$), 3-HKYN ($p < 0.0001$), KYNA ($p < 0.0001$) and AA ($p < 0.0001$) were increased in comparison with the control group. Also in saliva of uremic patients, the increase in concentration of KYN ($p < 0.0001$), 3-HKYN ($p < 0.0001$), KYNA ($p < 0.0001$) and AA ($p < 0.0001$) in comparison to the control group was noted.

Tab. 1. Baseline characteristics of healthy volunteers and patients with chronic renal failure

Parameter	n	Serum		
		Control	Prehemodialysis	p
Creatinine [mg/dl]	19	0.82 ± 0.4	8.42 ± 3.2	< 0.0001
Urea nitrogen [mg/dl]	19	11.9 ± 4.3	136.1 ± 43.7	< 0.0001
Albumin [g/dl]	19	4.8 ± 1.2	4.0 ± 0.4	0.0091

Discussion

The role of KYN and its metabolites in the saliva of uremic patients have not been studied yet. In the present study, we have observed a marked increase in KYN, KYNA, 3-HKYN and AA concentration in the saliva of patients with chronic renal failure. We have also found elevated concentration of these metabolites

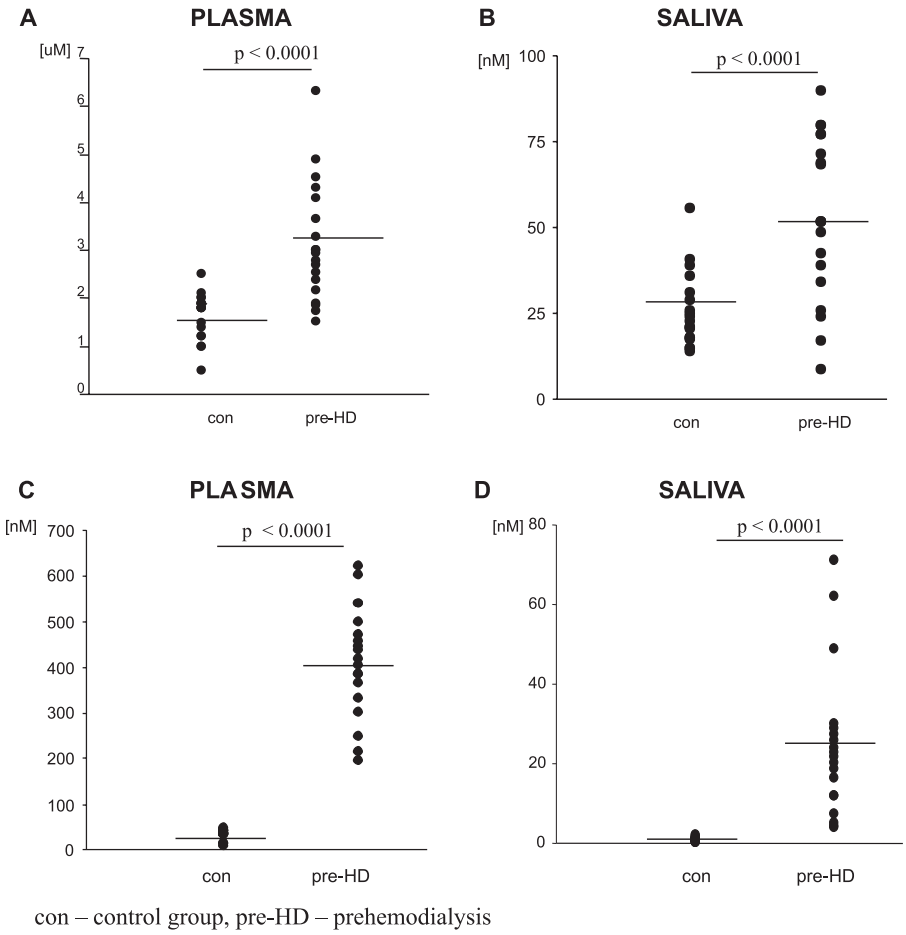

Fig. 1. The concentrations of kynurenine (KYN) in plasma (**A**) and saliva (**B**), and 3-hydroxykynurenine (3-HKYN) in plasma (**C**) and saliva (**D**) in patients with chronic renal failure

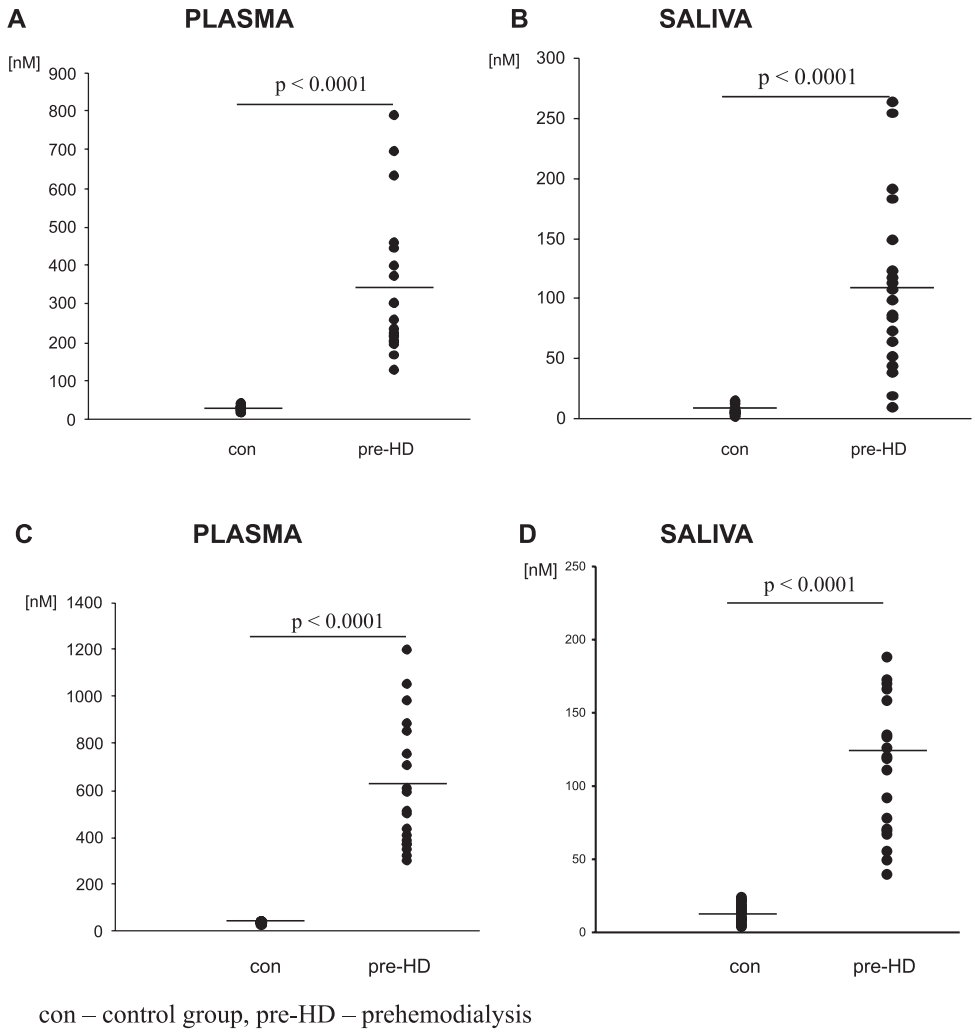

Fig. 2. The concentrations of kynurenic acid (KYNA) in plasma (**A**) and saliva (**B**), and anthranilic acid (AA) in plasma (**C**) and saliva (**D**) in patients with chronic renal failure

in plasma. Our observations are in line with the study of Saito and et al. [16] who demonstrated a marked increase in KYN and quinolinic acid concentrations in plasma of humans with renal insufficiency. An elevated plasma concentration of kynurenine pathway metabolites has been also observed in our previous study, in both, prehemodialysis and posthemodialysis patients with renal insufficiency [13].

The mechanism responsible for accumulation of kynurenine pathway metabolites in patients with chronic renal insufficiency is not known. We can hypothesize that elevated plasma KYN concentration is mainly caused by the increase in tryptophan 2,3-dioxygenase (TDO) activity and a decrease in kynureninases activity in the liver. This may lead to the increase in plasma level of the studied metabolites [16]. It can be speculated that the accumulation of KYN and its metabolites in saliva is caused by the entry of plasma kynurenines into the gingival crevicular fluid.

In our previous study, we have found an elevated concentration of KYN and KYNA in the saliva of hypertensive diabetic patients [3]. It is well established that diabetic patients are more susceptible to gingivitis and periodontitis than healthy subjects. These disorders are commonly considered to be oral complications of diabetes.

Oral symptoms in uremic subjects represent a relatively uncommon complication seen mostly in cases of end-stage chronic renal failure. They are characterized by the presence of painful plaques and crusts usually distributed on the buccal mucosa, dorsal or ventral surface of the tongue, gingival, lips and floor of

the month. Recently Ben-Zivi et al. [2] have demonstrated the increase in the oxidative stress burden in both, serum and saliva of diabetic and uremic patients.

In the literature, there are no data concerning the influence of KYN and its metabolites on the pathogenesis of uremic stomatitis lesions. The etiology of this pathology still remains unclear. It is known that IDO, which converted tryptophan into KYN, preferentially is induced by INF [7, 20]. KYN metabolites can induce apoptosis of thymocytes and terminally differentiated T helper cells, in particular Th1 colones [4]. It has been proven that T cell apoptosis was independent of Fas/Fas ligand interaction and was associated with activation of caspase-8 as well as release of cytochorme *c* from mitochondria. Other KYN metabolites, such as 3-hydroxyanthranilic acid and quinolinic acid induced caspase-8/FLICE activation and functional caspase-8 activity. Thus, caspase-8 might play a crucial role in the regulation of kynurenine-induced apoptosis in the organism [4, 11]. The immunoregulatory role of KYN metabolites may be important for maintaining peripheral lymphocyte homeostasis [4, 20]. So, we can speculate that the increase in KYN derivates in saliva can be responsible for local inflammatory changes in uremic patients.

In conclusion, the increased concentration of KYN, 3-HKYN, KYNA and AA in plasma and saliva of uremic patients in comparison with healthy volunteers suggests an altered metabolism of kynurenine in uremia. The accumulation of kynurenine pathway metabolites in saliva from uremic subjects may contribute to the certain symptoms observed in oral cavity.

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