

# Changes in Plasma Concentrations of Hypoxanthine and Uric Acid Before and After Hemodialysis

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**Introduction.** Purine metabolites constitute a major class of uremic toxins, and reliable characterization of which helps nephrologists to choose the most appropriate treatment for the patients individually. In the present study, we assessed plasma concentrations of hypoxanthine and uric acid as purine metabolites in patients on maintenance hemodialysis, before and after a dialysis session.

**Materials and Methods.** A total of 20 patients on maintenance hemodialysis were enrolled in this study. All of the patients underwent a routine 4-hour dialysis, as scheduled 3 times per week. Polysulfone membranes and bicarbonate dialysis solution were used in all dialysis sessions. Blood specimens were taken from the arteriovenous fistula immediately before and after one hemodialysis session, in order to measure plasma concentrations of hypoxanthine and uric acid by high-performance liquid chromatography, and to compare the predialysis and postdialysis values.

**Results.** Before hemodialysis, the mean plasma hypoxanthine and uric acid concentrations were  $18.93 \pm 8.28$   $\mu\text{mol/L}$  and  $44.16 \pm 22.88$   $\mu\text{mol/L}$ , respectively. After hemodialysis, these concentrations reduced to  $13.68 \pm 4.42$   $\mu\text{mol/L}$  and  $15.61 \pm 11.12$   $\mu\text{mol/L}$ , respectively. Hypoxanthine concentration had a 27.7% decrease after hemodialysis (mean difference,  $5.25 \pm 6.24$   $\mu\text{mol/L}$ ; 95% confidence interval, 2.32 to 8.10;  $P < .001$ ). Also, uric acid concentration decreased by 64.6% (mean difference,  $28.55 \pm 14.39$   $\mu\text{mol/L}$ ; 95% confidence interval, 21.81 to 32.28;  $P < .001$ ).

**Conclusions.** Plasma concentrations of hypoxanthine and uric acid are higher than normal before hemodialysis, and they decrease significantly after hemodialysis; however, both of them may be still higher than normal values.

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## INTRODUCTION

Uremic syndrome is the result of the retention of solutes, which are physiologically cleared by glomerular filtration. Some of the retained solutes are proven toxins. The identification, characterization, and analytical determination of toxins responsible for the adverse biological effects encountered in uremia and the knowledge of their

pathophysiological role is crucial for prevention and treatment of uremia in patients with end-stage renal disease (ESRD). The information obtained from these analyses will make it possible to evaluate existing therapeutic approaches and to define new prognostic markers for the removal of uremic toxins, and more importantly, it will allow the design of new specific removal strategies or

other interventions to decrease and even normalize plasma levels of uremic toxins.

Uric acid, xanthine, hypoxanthine, cytidine, and guanosine are the most important purine metabolites retained in uremia. The purine metabolites constitute a major class of uremic toxins, which can disturb vitamin D and calcitriol metabolization.<sup>1-4</sup> These may also play a role in the immunodeficiency state of patients on hemodialysis.<sup>5</sup> It is documented that poor appetite and weight loss are related to purine metabolites in patients with ESRD.<sup>6</sup> Elimination of some purine metabolites by hemodialysis shows no correlation with classic retained solutes in uremia, such as urea and creatinine.<sup>7</sup> It seems that acetate hemodialysis stimulates hypoxanthine production.<sup>8</sup> Furthermore, some studies suggest that hemodialysis with cuprophane dialysis membranes does not affect adenosine concentrations.<sup>8-19</sup> These studies revealed that only plasma level of hypoxanthine decreased after hemodialysis. On the other hand, concentrations of some adenosine metabolites are reported to increase after hemodialysis.<sup>17-20</sup>

Reliable characterization of uremic toxins and determination of their levels help nephrologists to choose the most appropriate treatment for patients on hemodialysis. Therefore, in the present study we assessed the plasma concentration of hypoxanthine and uric acid as purine metabolites in patients who received maintenance hemodialysis before and after dialysis.

## MATERIALS AND METHODS

We selected 24 patients with ESRD who were on chronic hemodialysis in our dialysis center. The study was approved by the Ethics Committee of Ahwaz Jondi Shapour University of Medical Sciences, and all patients gave written informed consent to participate in this study. Four patients had hypotension during the dialysis session and were excluded. Thus, we studied on 20 patients who were 12 men and 8 women with a mean age of  $61.6 \pm 9.9$  years (range, 41 to 69 years). Their mean duration of maintenance hemodialysis was  $32.0 \pm 18.0$  months (range, 16 to 44 months). The causes of ESRD were diabetic nephropathy in 8 patients, hypertensive nephropathy in 3, polycystic kidney disease in 2, unknown in 3, obstructive nephropathy in 3, and lupus nephritis in 1. All of the patients were on 4-hour dialysis sessions, 3 times per week. Polysulfone dialysis membranes

were used in all dialysis sessions, and the dialysis solution contained sodium, 133 mmol/L; potassium, 1.0 mmol/L; magnesium, 0.5 mmol/L; calcium, 1.5 mmol/L; chloride, 105.0 mmol/L; bicarbonate, 35.7 mmol/L; and glucose, 5 mmol/L. Vascular access was either native radial arteriovenous fistula ( $n = 12$ ) or graft polytetrafluoroethylene fistula ( $n = 8$ ).

Blood samples were taken from the arteriovenous fistula immediately before and 2 hours after a hemodialysis session. The samples were added to tubes containing 1.3 mol of perchloric acid at a volume equal to that of the added blood. The tubes were then centrifuged and the supernatants were neutralized with 3 M of  $\text{NaH}_2\text{PO}_4$ . Plasma concentrations of hypoxanthine and uric acid were measured by high-performance liquid chromatography, using an automated system on a  $4 \times 125$ -mm Eurosphere-100C18 column (Eurosphere 100 C18, Knauer, Berlin, Germany) and a detector (UV/Visible, Cecil Instruments, Cambridge, UK). Uric acid and hypoxanthine were both from Sigma Chemical Co (St Louis, Missouri, USA). The reference ranges for uric acid were 15  $\mu\text{mol/L}$  to 33  $\mu\text{mol/L}$  for women and 18  $\mu\text{mol/L}$  to 41  $\mu\text{mol/L}$  for men. Sample injection, data analysis, and producing standard curves were tested and adjusted according to the instructions.<sup>21</sup> Calibration curves were prepared by treating the standard mixtures in the same way as plasma samples. Calibration curves suitable for the analysis of plasma were linear ( $r^2 > 0.95$ ) with limits of detection from 10 ng/mL to 100 ng/mL. Both intraday and interday relative standard deviations were lower than 10%.

The results were analyzed using Data Control statistical package (Cecil Inc, Ohio, USA). Data on continuous variables were summarized as the mean  $\pm$  standard deviation. Predialysis and postdialysis values were assessed using the Wilcoxon signed rank test for continuous paired variables. A value of  $P$  less than .05 was considered significant.

## RESULTS

Twenty patients were enrolled in this study and their blood samples were taken before and after a 4-hour dialysis session. The mean  $Kt/V$  was  $1.13 \pm 0.11$  (range, 1.0 to 1.22). Before hemodialysis, the mean serum creatinine level was  $4.66 \pm 0.52$  mg/dL (range, 3.9 mg/dL to 5.4 mg/dL), and the

Plasma Hypoxanthine and Uric Acid Concentrations in Plasma of Patients on Hemodialysis

Electrolyte	Before hemodialysis	After Hemodialysis	P
Hypoxanthine, $\mu\text{mol/L}$	18.93 $\pm$ 8.28 (16.9 to 19.6)	13.68 $\pm$ 4.42 (10.4 to 15.3)	< .001
Uric acid, $\mu\text{mol/L}$	44.16 $\pm$ 22.88 (37.1 to 49.1)	15.61 $\pm$ 11.12 (14.0 to 17.9)	< .001

mean blood urea nitrogen level was  $98.0 \pm 22.4$  mg/dL (range, 70 mg/dL to 126 mg/dL).

Table 1 represents hypoxanthine and uric acid concentrations in plasma of the patients before and after hemodialysis.

Plasma hypoxanthine concentration significantly reduced after hemodialysis (27.7% on average; mean difference,  $5.25 \pm 6.24$   $\mu\text{mol/L}$ ; 95% confidence interval, 2.32 to 8.10;  $P < .001$ ). Also, uric acid concentration decreased significantly after hemodialysis (64.6% on average; mean difference,  $28.55 \pm 14.39$   $\mu\text{mol/L}$ ; 95% confidence interval, 21.81 to 32.28;  $P < .001$ ). Before hemodialysis, uric acid and hypoxanthine levels were within normal values in 1 patient. Two hours after hemodialysis, uric acid and hypoxanthine levels were still higher than normal values in 3 and 2 patients, respectively.

In the chromatographic peaks dimensions, some of the metabolites changed in concentration before and after hemodialysis. Changes in uric acid and hypoxanthine concentrations which were identified and quantified could also be clearly seen. Furthermore, some compounds were observed by our chromatographic method, which were unknown.

## DISCUSSION

Purine nucleotide metabolism changes in chronic kidney disease. Purine metabolites are retained in patients with ESRD and lead to some uremic symptoms. To better understand the role of hemodialysis on purine metabolites in patients with ESRD, we assessed the plasma concentration of both hypoxanthine and uric acid before and after hemodialysis. Significant decreases in plasma levels of hypoxanthine and uric acid were documented after hemodialysis.

Phosphorilation of adenosine into adenine is the first step of purine degradation in human. Adenine is deaminated rapidly into inosine, which is converted to hypoxanthine. Then, hypoxanthine is converted to xanthine by xanthine oxidase. Finally, xanthine is metabolized to uric acid, the final product of purine degradation in humans.

Uric acid, xanthine, and hypoxanthine are the most important purine metabolites retained in uremia. Xanthine and hypoxanthine have been implicated as modulators of neurotransmission and may be related to poor appetite and weight loss.<sup>6</sup> Both xanthine and hypoxanthine induce vasoconstriction and disturb endothelial barriers.<sup>22,23</sup> Xanthine also acts as a substrate for xanthine oxidase and enhances superoxide generation, which plays a major role in microvascular dysfunction and exerts direct tissue damage, leading to lipid peroxidation. A study by Linas and coworkers found that xanthine oxidase depletion improved kidney function after reperfusion.<sup>24</sup> Also, plasma concentrations of xanthine and hypoxanthine are connected with delayed graft function in kidney transplant recipients.<sup>25</sup>

Uric acid is a small water-soluble compound that is removed by hemodialysis from plasma in a similar way as urea, but its removal from the intracellular compartment is by far not as efficient as that of urea.<sup>26,27</sup> Epidemiologic studies have also found that hyperuricemia is an independent risk factor of kidney dysfunction in the general population, as well as patients with hypertension, diabetes mellitus, and chronic kidney disease.<sup>28-31</sup> In addition, hyperuricemia has been found to accelerate kidney disease in the remnant kidney model and to accelerate experimental cyclosporine nephropathy.<sup>32,33</sup> The importance of these pathways is suggested by a recent prospective study, in which lowering uric acid in individuals with hyperuricemia and kidney dysfunction was associated with improved blood pressure control and slower progression of kidney disease.<sup>34</sup> It should also be noted that uric acid may contribute to vascular disease.<sup>35</sup>

We found high concentrations of hypoxanthine and uric acid in plasma of patients with ESRD before hemodialysis, which was in agreement with findings of the previous reports.<sup>8,9</sup> In our patients, the concentrations of plasma hypoxanthine and uric acid decreased significantly after hemodialysis. However, their concentrations were higher than

normal after hemodialysis, in spite of relatively high uric acid and hypoxanthine removal. Bullo and associates found the same results.<sup>8</sup> They reported that in spite of twofold decrement, blood concentration of hypoxanthine was higher than normal even after 24 hours of hemodialysis. In contrast to their patient, however, our patients were on dialysis by bicarbonate dialysis solution. In another study, Bober and coworkers reported that purine metabolites decreased significantly after hemodialysis in patients who were on dialysis with dialysis solutions either containing or not containing glucose. They showed that glucose-containing dialysis solutions could influence in the decrement of purine metabolites after hemodialysis.<sup>9</sup> Tekkanat and colleagues showed that purine metabolites increased after hemodialysis with acetate dialysate.<sup>10</sup> In accordance with these studies, we found that purine metabolites concentrations were higher than normal both before and after hemodialysis. This observation could be due to either incomplete hypoxanthine and uric acid clearance by hemodialysis or the stimulated endogenous synthesis or impaired degradation during a hemodialysis session.

High plasma levels of hypoxanthine and uric acid even after hemodialysis could also be explained by occurrence of ischemic events during hemodialysis. Ischemic events during hemodialysis enhance adenosine nucleotide degradation, which lead to adenosine triphosphate hydrolysis, anaerobic glycolysis, progressive reduction of the iron-sulfur proteins associated with nicotinamide adenosine dinucleotide dehydrogenase, and proteolytic conversion of xanthine dehydrogenase to xanthine oxidase.<sup>13-15</sup> All together, these mechanisms cause adenosine triphosphate degradation and increase hypoxanthine and uric acid production during ischemia. The influence of ischemic events on adenosine triphosphate degradation has been documented in conditions other than hemodialysis, as well. Woolliscroft and colleagues demonstrated that urine oxypurine-creatinine ratio increased after hypotensive events in patients with chronic heart failure, liver diseases, etc.<sup>16</sup>

Intradialytic hypotension is one of the most prominent features of ischemic events in patients with ESRD during hemodialysis. Shinzato and coworkers assessed the plasma level of purine metabolites in patients on hemodialysis during

intradialytic hypotension episodes.<sup>17</sup> They showed that purine metabolites increased during hypotension. They showed also that caffeine as an adenosine antagonist could attenuate intradialytic hypotension episodes. Likewise, Imai and associates showed that FK352, a selective A1 antagonist, improved intradialytic hypotension.<sup>18</sup> These clinical studies showed that adenosine and its metabolites incorporate in hemodialysis complications.

## CONCLUSIONS

We conclude that plasma concentration of hypoxanthine and uric acid are higher than normal before hemodialysis in patients with ESRD and their plasma levels decrease significantly after hemodialysis, but they are still higher than normal values.

## CONFLICT OF INTEREST

None declared.

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