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Plasma oxalate levels in prevalent hemodialysis patients and potential implications for ascorbic acid supplementation

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ABSTRACT

Objectives: Ascorbic acid (AA) supplementation may increase hemoglobin levels and decrease erythropoiesis-stimulating agent dose requirement in patients with end stage renal disease (ESRD). While plasma AA levels >100 μM may be supratherapeutic, levels of at least 30 μM may be needed to improve wound healing and levels may need to reach 70 μM to optimize erythropoiesis. Of concern, oxalate (Ox), an AA metabolite, can accumulate in ESRD. Historically, if plasma Ox levels remain ≥30 μM, oxalosis was of concern. Contemporary hemodialysis (HD) efficiencies may decrease the risk of oxalosis by maintaining pre-HD Ox levels <30 μM. This study focuses on the plasma Ox levels in HD patients.

Design and methods: A prospective, observational study of 197 HD patients with pre-HD AA levels and pre-HD and post-HD Ox levels.

Results: Mean plasma Ox levels decreased 71% during the intradialytic period (22.3 ± 11.1 μM to 6.4 ± 3.2 μM, $P < 0.001$). In regression analysis, pre-HD plasma AA levels ≤100 μM were not associated with a pre-HD plasma Ox level ≥ 30 μM, even if ferritin levels were increased. Pre-HD plasma Ox levels ≥20 or ≥30 μM were not associated with lower cumulative 4-year survival.

Conclusions: Pre-HD plasma AA levels up to 100 μM in HD patients do not appear to be associated with an increased risk of developing secondary oxalosis, as the corresponding pre-HD plasma Ox level appears to be maintained at tolerable levels.

1. Introduction

In end stage renal disease (ESRD), both anemia and the escalation of erythropoiesis-stimulating agent (ESA) dose in response to low hemoglobin levels are associated with increased morbidity [1] and mortality [2]. Preliminary evidence indicates that ascorbic acid (AA, vitamin C) supplementation may provide a strategy to both treat anemia and allow lowering of the ESA dose [3–5].

Hemodialysis (HD) patients commonly have plasma AA concentrations (P[AA]) below thresholds [6] that may prevent bleeding [7], optimize wound healing [8], and support effective erythropoiesis [9]. When subjects with normal kidney function are administered high doses of AA, they are unable to achieve a P[AA] greater than 100 μM, because of urinary AA loss [10]. This urinary loss may indicate that a P[AA] up to 100 μM is sufficient to support normal physiological processes.

Concern for the development of de novo secondary oxalosis has precluded heretofore the use of AA as an adjuvant to ESA therapy [4,11]. Oxalate (Ox), an AA-metabolite, may accumulate when the glomerular filtration rate decreases to <30 mL/min [12]. When plasma Ox concentration (P[Ox]) reaches 30 μM, Ox has the potential to precipitate with calcium [13,14]. These precipitates may then deposit pathologically in organs [15], leading to secondary oxalosis [16]. HD [17] and peritoneal dialysis [18] provide alternative routes of Ox elimination. However, even when receiving 100 mg or less of AA/day, HD patients in previous studies could attain a P[Ox] of 30 μM or higher [17,19–21]. Studies to

Abbreviations: AA, ascorbic acid; ESRD, end stage renal disease; HD, hemodialysis; Ox, oxalate; P[AA], plasma ascorbic acid concentration; P[Ox], plasma oxalate concentration.

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evaluate this potential of oxalosis developing were performed before the adoption of current dialysis adequacy targets and before the use of high-flux dialyzers.

We previously reported on pre-HD plasma AA levels in patients on maintenance HD [6]. The present study details the pre-HD and post-HD plasma Ox levels that were simultaneously measured in this cohort. The combined results address the effect of contemporary HD on P[Ox], the potential significance of increased pre-HD P[Ox] in prevalent patients, the risk of surpassing a pre-HD P[Ox] ≥ 30 μM at a pre-HD P[AA] ≥ 30 μM or ≥ 70 μM, and other factors associated with reaching this plasma Ox threshold.

2. Methods

2.1. Study design, study population, and laboratory measurements

We conducted a prospective, observational study in which pre-HD and post-HD P[Ox] were measured in a prevalent HD population. Adult patients on maintenance HD at four outpatient HD clinics were considered eligible for inclusion. Patients were excluded if they were pregnant, less than 18 years old, unable to provide informed consent, or had been on maintenance HD for less than 2 months. High-flux dialyzers (Baxter CT 190G or Exeltra 190; Fresenius F160NR, F180NR, or F200NR; Gambro Revaclear 1170 or Revaclear Max 1490) were used during all HD treatments. All patients provided informed consent to participate in the study, which was approved by the Institutional Review Board of Cooper University Hospital.

Plasma samples were collected before and immediately after a Monday or Tuesday dialysis session, if the patient had undergone HD on the preceding Friday or Saturday, respectively.

Using an established protocol for plasma Ox measurement, venous blood (5 mL) was collected into a lithium-heparin tube and placed on ice [22,23]. Briefly, within 30 min, the tubes were centrifuged at 2500 rpm for 10 min. Next, 2 mL of plasma was added to an empty Centrisart tube (10,000 MW). The inner liner segment containing 40 μL of 2 M hydrochloric acid was then inserted into the Centrisart tube. This tube was then centrifuged for 30 min at 2500 rpm. An aliquot of the solution from the inner liner tube was frozen at −80 °C until assayed for P[Ox]. Plasma samples were sent on dry ice overnight to Lowell, MA. Plasma samples were assayed in duplicate for P[Ox] by HPLC with detection by ion chromatography as previously described [22,23]. The HPLC consisted of a Dionex IS-1000 ion chromatograph with a 25 × 0.46 cm AS-11 ion exchange column. The mobile phase was 8 mM NaOH, at a flow rate of 1.2 mL/min. Samples were diluted 1/10 with 1 0.1 M boric acid before analysis, and 25 μL was injected on the HPLC. The method in our laboratory had a precision of ± 5% and was linear in the 5–200 μM range.

Following an established protocol for plasma AA measurement [6], within 30 min of collection into a lithium-heparin tube that had been placed on ice, venous blood (5 mL) was centrifuged at 2500 rpm for 10 min. An aliquot of the plasma (200 μL) was diluted with an equal volume of 10% metaphosphoric acid and frozen at −80 °C until assayed for AA. These plasma samples were sent on dry ice overnight to Lowell, MA. They were assayed in duplicate for AA by HPLC on a Beckmann C18 column (250 mm × 4.6 mm) with electrochemical detection [6]. The detection limit for this method was 1 μM and had a precision of ± 2%.

Charts were abstracted for demographic variables, medical history, HD treatment parameters and routine clinical laboratory results. Potassium (determined by using an ion selective electrode) and albumin (measured by light absorbance utilizing bromcresol green reagent) analyses were performed on an Olympus AU 5400. Ferritin (detected by chemiluminescence immunoassay) analysis was performed on an Advia Centaur. The CMS Medical Form 2728 was reviewed to determine ESRD cause.

2.2. Statistical analysis

All mean and median values for continuous data are reported with standard deviations (SD) and interquartile ranges (IQR), respectively, as appropriate to the distribution of the data.

We used a paired *t*-test to assess the comparison of mean pre-HD and post-HD P[Ox]. Pearson correlation was used to examine the relationship between continuous variables and pre-HD P[Ox]. Pearson chi square compared the percentage difference of a spKt/V < 1.5 and a potassium <5 mmol/L in patients with a pre-HD P[Ox] ≥ 30 μM as compared to those patients with a pre-HD P[Ox] < 30 μM. A one-way ANOVA was used to compare pre-HD P[Ox] and mean plasma albumin concentrations over 3 discrete ferritin ranges. The corresponding median pre-HD P[AA] over these ranges was compared by utilizing the Kruskal-Wallis test. Odds ratios (OR) were calculated to assess the risk of attaining a pre-HD P[Ox] ≥ 30 μM at pre-HD P[AA] thresholds of ≥ 30 μM or ≥ 70 μM. Binomial logistic regression analysis incorporated the following independent variables: spKt/V < 1.5, potassium <5 mmol/L, median dialysis vintage [≤ or >1012 days], ferritin ≥ 1124 pmol/L or ≥ 2472 pmol/L, and pre-HD P[AA] ≥ 30 μM or ≥ 70 μM. We used an independent *t*-test to compare the mean pre-HD P[Ox], ferritin, and spKt/V and the Mann-Whitney *U*-test to compare the median pre-HD P[AA] and patient vintage in patients with CAD, CHF, and/or PVD to the corresponding values seen in patients without these corresponding conditions.

At the time of plasma sample collection, information regarding AA supplementation (mg/day) was obtained [6]. Spearman Rho correlation was used to assess the relation between AA supplementation and pre-HD P[AA]. Pearson chi square compared the percentage difference of attaining a pre-HD P[AA] > 100 μM in patients who took varying degrees of AA supplementation.

Lastly, Kaplan-Meier curves, utilizing log-rank tests, were compared for the cutpoints of a pre-HD P[Ox] of ≥ 20 and 30 μM, levels approximately 2× and 3× normal [24], respectively. Patients were censored from analysis at the time of transplantation, loss to follow up, or withdrawal from HD.

Statistical analysis was performed with SPSS (PASW) version 22 (IBM corporation, Armonk, NY).

3. Results

3.1. Oxalate kinetics

Two hundred three patients were enrolled between May and October 2011. Six patients had incomplete plasma sample collection for Ox. Results are reported for the remaining 197 patients. Single pool Kt/V for the study population was 1.8 ± 0.3 (Table 1). Mean P[Ox] decreased 71% from 22.3 ± 11.1 μM (range 2.8–63.6) before dialysis to 6.4 ± 3.2 μM (range 1.3–20.6) after dialysis ($P < 0.001$).

3.2. Relationship of pre-HD P[Ox] to pre-HD P[AA]

There was a moderate association between pre-HD P[AA] and pre-HD P[Ox] ($r = 0.581$, $P < 0.001$) (Fig. 1A). As a P[AA] greater than 100 μM may be 1) supratherapeutic and 2) detrimental as HD patients may be more likely to develop a P[Ox] ≥ 30 μM [21], this association was also examined in only the patients with a pre-HD P[AA] ≤ 100 μM. This relationship was attenuated somewhat in this subgroup ($r = 0.236$, $P = 0.003$) (Fig. 1B).

In the whole cohort, the likelihood of having a pre-HD P[Ox] ≥ 30 μM was higher when patients had a pre-HD P[AA] of ≥ 30 μM (OR 4.7, 95% confidence interval [95% CI] 1.7–16.1, $P = 0.001$) or ≥ 70 μM (OR 11.3, 95% CI 4.8–27.2, $P < 0.001$). If we limited the analysis to patients with a pre-HD P[AA] ≤ 100 μM, the likelihood of a pre-HD P[Ox] ≥ 30 μM was not increased among patients with a pre-HD P[AA] ≥ 30 μM

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