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CLINICAL RESEARCH

Asymmetric Dimethylarginine (ADMA) Levels Are Lower in Hemodialysis Patients Treated With Paricalcitol

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Introduction: Chronic kidney disease is a major public health problem. In the last decade, it has been shown that the early stages of chronic kidney disease are associated with an inflammatory condition involving an increased risk of cardiovascular morbidity and long-term mortality. In patients with chronic kidney disease and more specifically those on hemodialysis, cardiovascular events are the most common cause of death. Asymmetric dimethylarginine (ADMA) is an endogenous inhibitor of nitric oxide synthase and may be an independent risk factor for endothelial dysfunction and cardiovascular disease.

Methods: We performed a cross-sectional analysis to identify factors that were associated with ADMA such as certain medications related to cardiovascular disease in dialysis patients.

Results: Patients who were treated with paricalcitol had significantly lower levels of ADMA (0.21 $\pm$ 0.19 μmol/l) compared with those not treated with paricalcitol (0.42 $\pm$ 0.35 μmol/l) ($P = 0.00027$). Dividing ADMA levels by quartiles, patients treated with paricalcitol were less likely to have very high level ADMA ($P = 0.014$), whereas there were no significant differences with other medications. Higher dose of paricalcitol was also related to lower levels of ADMA noting an inverse correlation ($r = –0.36$, $P = 0.013$).

Discussion: Hemodialysis patients treated with paricalcitol presented significantly decreased ADMA levels compared with those who did not receive this treatment. Possible beneficial effects in terms of cardiovascular morbidity and mortality by paricalcitol and its association with ADMA and nitric oxide synthesis are unknown. Studies to confirm this effect and determine the underlying pathophysiological mechanism are necessary.

Kidney Int Rep (2017) **2,** 165–171; http://dx.doi.org/10.1016/j.ekir.2016.10.002
KEYWORDS: asymmetric dimethylarginine; dialysis; ESRD; hemodialysis; paricalcitol

Asymmetric dimethylarginine (ADMA) is an endogenous nitric oxide synthase inhibitor that may be an independent risk factor for endothelial dysfunction and cardiovascular disease.[1] Patients with and without established coronary heart disease and elevated levels of serum ADMA have an increased risk of acute coronary events compared with individuals with lower ADMA levels.[2–4] ADMA, which is significantly increased in end-stage renal disease (ESRD),[5] is an endogenous inhibitor of nitric oxide.[6,7] Inhibition of nitric oxide synthesis in patients with ESRD may cause vasoconstriction and hypertension, thereby resulting in adverse cardiovascular outcomes.[8–11] In hemodialysis patients, plasma ADMA is a strong and independent predictor of overall mortality and cardiovascular outcome.[9]

Paricalcitol has demonstrated effects on endothelial function and suppressing inflammation decreasing levels of high-sensitivity C-reactive protein, tumor necrosis factor-α, and interleukin-6 in hemodialysis patients.[12] High ADMA levels are a consequence of endothelial dysfunction[1] and the effects of paricalcitol on ADMA levels have not been reported.

Certain drugs such as proton pump inhibitors have been associated with the elevation of asymmetric

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Received 23 March 2016; revised 6 October 2016; accepted 13 October 2016; published online 18 October 2016

dimethylarginine levels,[13] whereas other drugs have been associated with a reduction of ADMA levels in several reports, such as certain angiotensin converting enzyme inhibitors and angiotensin receptor blockers,[14–16] metformin,[17] rosiglitazone,[17,18] or rosuvastatin.[19]

We report a study designed to evaluate the relationship between levels of ADMA with survival in hemodialysis patients and a cross-sectional analysis to study the association between levels of ADMA and the use of drugs that have demonstrated a reduction of cardiovascular disease in dialysis patients, such as statins, aspirin, angiotensin converting enzyme inhibitors and/or angiotensin receptor blockers, and paricalcitol.

METHODS

Study Design, Setting, and Patients

We performed a cross-sectional observational study in patients on chronic hemodialysis to evaluate the association of ADMA with medication as well as a prospective study to evaluate ADMA with mortality. The study was performed in the Hospital Universitario de Gran Canaria Doctor Negrin, Gran Canaria, Spain, between April 2011 and April 2014. Ninety-three patients who underwent chronic hemodialysis treatment (62.4% men and 37.6% women) were randomly selected from a total of 231 patients. All patients were being treated 3 times a week with standard bicarbonate dialysis (Na^+ 138 mmol/l, HCO_3^- 35 nnol/l, K^+ 1.5 mmol/l, Ca^{2+} 1.25 mmol/l, Mg^{2+} 0.75 mmol/l) with 1.7 or 2.1 polysulfone membrane dialyzers.

Patients continued with their usual treatment prescribed for chronic diseases previously diagnosed. They had no changes on medication related to CKD bone mineral disease for 2 months before the serum for determination of ADMA levels was collected. No changes were therefore made of paricalcitol or calcitriol from 2 months before. No patients had treatment with calcitriol or alphacalcidol. There were no significant differences between quartiles attending to cinacalcet or phosphate binders (calcium and non–calcium-based phosphate binders).

The protocol adhered to the ethical guidelines of our hospital and this study was evaluated and accepted by the Clinical Research Ethics Committee of the Hospital Universitario de Gran Canaria Doctor Negrin, which meets BPC (CPMP/ICH/135/95) standards and Spanish laws (R.D. 223/2004). All patients enrolled in the study received exhaustive information, and were asked for their participation, signing the corresponding consent.

A review of medical records was conducted, as well as computer databases used in the Nephrology Department to collect data, including demographic, anthropometric, physical examination, vital signs, and biochemical data. At 36 months, a review of death records was conducted. Finally, we proceeded to the statistical analysis of the results.

The variables analyzed are defined in Tables 1 and 2.

Laboratory

Samples of peripheral venous blood were obtained, the first dialysis day of the week (Monday or Tuesday), after a minimum fasting period of 8 hours. Blood samples were sent to the reference laboratory for biochemical determinations. The samples for ADMA determination were kept at –80°C until the analysis of ADMA was performed.

Table 1. Baseline characteristics of patients including totals and quartiles of ADMA levels (μmol/l)

	Total	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P* value
Age (yr)	66 (19)	65 (20)	64 (10)	70 (19)	74 (19)	0.15
Sex (men)	Men: 58 (62.4%)	15 (62.5)	14 (60.9)	16 (69.6)	13 (56.5)	0.86
Dry weight (kg)	71.0 ± 13.4	68.3 ± 13.5	70.8 ± 11.8	75.7 ± 12.1	69.5 ± 69.5	0.26
BMI (kg/m^2)	25.7 ± 4.7	24.1 ± 4.3	25.7 ± 5.0	26.9 ± 4.0	26.2 ± 5.0	0.19
BMI ≥ 30 kg/m^2	Yes: 16 (17.2%)	2 (8.3)	4 (17.4)	4 (17.4)	6 (26.1)	0.475
Time in HD (mo)	53.1 (57.9)	46 (80)	46.7 (44.1)	58.2 (61.2)	53.9 (54.8)	0.48
Central venous catheter	15 (16.1%)	1 (4.2)	4 (17.4)	4 (17.4)	6 (26.1)	0.21
Charlson Comorbidity Index	4 (2)	3.5 (2)	4 (2)	5 (2)	3.5 (2)	0.46
Ethiology of CKD	Diabetic nephropathy: 35 (37.6%) Nephroangioesclerosis: 6 (6.5%) Polycystic kidney disease: 10 (10.8%) Glomerulonephritis: 13 (14%) Others: 21 (22.6%) Unknown: 8 (8.6%)	10 (41.7) 1 (4.2) 4 (16.7) 3 (12.5) 5 (20.8) 1 (4.2)	7 (30.4) 0 (0) 3 (13) 5 (21.7) 6 (26.1) 2 (8.7)	8 (34.8) 4 (17.4) 2 (8.7) 3 (13) 4 (17.4) 2 (8.7)	10 (43.5) 1 (4.3) 1 (4.3) 2 (8.7) 6 (26.1) 1 (4.3)	0.82
DM	Yes: 42 (45.2%)	10 (41.7)	11 (47.8)	9 (39.1)	12 (52.2)	0.82
ACEi/ARBs	22 (23.7%)	9 (37.5)	4 (17.4)	5 (21.7)	4 (17.4)	0.348
Paricalcitol	37 (39.8%)	14 (58.3)	11 (47.8)	8 (34.8)	4 (17.4)	0.026
Coronary arterial disease	23 (25.3%)	5 (20.8)	5 (22.7)	6 (26.1)	7 (31.8)	0.87

Data are shown as mean (SD), median (interquartile range), or number of cases (percentage frequency), as appropriate.
ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; BMI, body mass index; CKD, chronic kidney disease; DM, diabetes mellitus; HD, hemodialysis.

Table 2. Differences between groups with and without paricalcitol treatment

	Total (n = 93)	Non–paricalcitol-treated group (n = 56)	Paricalcitol-treated group (n = 37)	*P* value
Categorical variables				
Sex (men)	57 (61.3)	34 (59.6%)	23 (40.4%)	1
Diabetes	42 (41.2)	31 (73.8%)	11 (26.2%)	0.019
ARB/ACE inhibitors	22 (23.7)	13 (59.1%)	9 (40.9%)	1
Aspirin	62 (66.7)	40 (64.5%)	22 (35.5%)	0.27
Statins	65 (69.9)	39 (60%)	26 (40%)	1
Blood pressure medication	56 (60.2)	33 (58.9%)	23 (41.1%)	0.83
Continuous variables				
Age (yr)	64.7 ± 13.1	66.2 ± 13.0	62.4 ± 13.1	0.17
BMI (kg/m^2)	25.7 ± 4.7	25.6 ± 4.6	25.9 ± 4.8	0.78
Duration of dialysis treatment (mo)	65.3 ± 43.9	58 ± 33.4	74.7 ± 55.2	0.12
Basal glucose (mg/dl)	110.4 ± 65.7	111.8 ± 57.5	108.4 ± 77.1	0.82
Cholesterol (mg/dl)	143.3 ± 31.6	146.4 ± 34.7	138.7 ± 26.2	0.26
Albumin (g/dl)	3.8 ± 0.3	3.8 ± 0.4	3.8 ± 0.3	0.45
Ca (mg/dl)	9.2 ± 0.6	9.2 ± 0.5	9.1 ± 0.7	0.36
P (mg/dl)	5.1 ± 1.4	5.2 ± 1.6	4.9 ± 1.1	0.31
PTHi (pg/ml)	445.6 ± 394.8	417.2 ± 375.7	487.5 ± 423.2	0.41
PTHi nichols	310.8 ± 307.8	292.8 ± 293	336.3 ± 330.1	0.52
25 OH vitamin D (ng/dl)	16.6 ± 12.5	14.4 ± 7.9	19.8 ± 16.8	0.08
Homocysteine (μmol/l)	31.9 ± 22.9	32 ± 22.2	31.76 ± 24.3	0.96
Kt/V Daugirdas	1.5 ± 0.4	1.6 ± 0.4	1.5 ± 0.4	0.76

Data are shown as mean (SD), median (interquartile range) or number of cases (percentage frequency), as appropriate.
ACE, angiotensin converting enzyme; ARB, angiotensin receptor blockers; BMI, body mass index; PTH, parathyroid hormone.

Procedures

ADMA levels were determined by high-performance liquid chromatography (HPLC), a method using derivatization with o-phthalaldehyde based on the procedure described by Chu *et al.*,[20] after removal of plasma samples with carboxylic acid solid-phase extraction cartridges. Increasing concentrations of ADMA were spiked into the pooled blank plasma to achieve the concentrations of ADMA. After the addition of internal standard, standards were subjected to solid-phase extraction, derivatization, and chromatography as already mentioned. Because there were endogenous ADMA, symmetric dimethylarginine, and arginine in the blank plasma, the actual peak heights of spiked standards were calculated by subtracting the peak heights of analytes in the blank plasma from the peak heights of analytes in the spiked standards. Calibration curves were constructed by plotting the ratios of the actual peak height of analyte over internal standard versus analyte concentration. For the analysis of plasma samples, a calibration curve was performed in each run. Paricalcitol was administrated in dialysis sessions and by i.v.

Reagents

ADMA, o-phtaldialdehyde, 2-mercaptoethanol, trichloroacetic acid, triethylamine, boric acid, and sodium acetate were purchased from Sigma Chemical Co. (St. Louis, MO). Acetonitrile (HPLC grade), methanol (HPLC grade), potassium hydroxide (KOH), and tetrahydrofuran were from Merck-España (Madrid, Spain).

Equipment

For our study, we used a VARIAN PS230 chromatograph (1644 series) and reverse phase-HPLC.

Statistical Analysis

The sample was described by means of frequencies and percentages for qualitative variables and mean ± SD or median (interquartile range [IQR] = Q3–Q1) for quantitative variables, depending on the setting of the variables to the normal distribution. Survival was evaluated using Cox regression analysis using ADMA variable raw and adjusted for other variables, including age variable dependent on time. No variable selection algorithm was used. The Kaplan-Meier method was applied to evaluate the survival comparing the group consisting of the upper quartile of ADMA ($P > 75$, Q4) against the rest. The curves were compared with the log-rank test. Death probability at the end of the study using a logistic regression model was calculated. Time to event was considered from the moment of ADMA determination until event (death) or end of the study. No patients were lost to follow-up. To evaluate the association between paricalcitol with categorical variables, the Fisher exact test was used. The relationship between ADMA and other variables was explored by comparing ADMA means by analysis of variance (for more than 2 groups), the *t* test, or the Mann-Whitney *U* test (for 2 variables, according to the distribution). In the *t* test, the Welch correction was performed when the Levene homogeneity test of variance was significant. Multiple linear regression was also performed to analyze ADMA with other variables to study effect measure modifiers and confounders. Likewise a robust regression method (bootstrapping) was performed to compare with the multiple linear regression analysis.

To evaluate the association of ADMA with continuous quantitative variables, scatter plot diagrams were done and Pearson correlation coefficient was performed when there was linearity in the relationship. Finally, ADMA was categorized as quartiles, comparing the upper quartile with the highest concentration of ADMA to the rest. The significance level was established at $\alpha = 0.05$.

The statistical program used was R Core Team (2014).

RESULTS

Ninety-three patients who underwent chronic hemodialysis treatment (62.4% male) were randomly selected from a total of 231 patients, and had an average age of 64.7 ± 13.1 years. Of them, 45.2% were diabetic, and diabetic nephropathy was the most frequent cause of ESRD (37.6%). The median time in dialysis treatment was 53.1 months (IQR = 57.9). The baseline characteristics and frequency of medications are shown in Table 1.

Plasma ADMA concentrations were measured, showing a median concentration of 0.2 μmol/l (IQR = 0.48). Patients treated with paricalcitol had significantly lower ADMA levels (0.21 ± 0.19 μmol/l vs. 0.42 ± 0.35 μmol/l; $P = 0.00027$), as shown in Figure 1.

Patients were also divided into 4 quartiles according to ADMA levels. The patients in the highest ADMA concentration quartile were compared with those in the other 3 quartiles (median of 0.64 μmol/l, IQR = 0.06 vs. median of 0.18 μmol/l, IQR = 0.18). We compared the number of patients in the highest quartile (Q4) and the treatment patients received, noting that those who received paricalcitol were significantly less likely to be in the very high level ADMA group ($P = 0.014$). We did not observe statistical differences between ADMA levels and the use of angiotensin receptor blocker/angiotensin converting enzyme inhibitors ($P = 0.15$), statins ($P = 0.25$), or aspirin ($P = 0.93$). The distribution of patients treated with paricalcitol with lower levels of plasma ADMA compared with those not treated with paricalcitol who had higher levels is represented in Figure 2.

The clinical and laboratory characteristics of patients who were treated with paricalcitol (n = 37) and patients who were not treated with paricalcitol (n = 56) are shown in Table 2. There were no significant differences in age, sex, treatment with angiotensin receptor blockers/angiotensin converting enzyme inhibitors, aspirin or statins, body mass index, time on dialysis, cholesterol, albumin, serum calcium, phosphate, parathyroid hormone (PTH), or vitamin D levels between patients who were or were not on paricalcitol at the time that the ADMA levels were obtained. Patients on paricalcitol had a lower prevalence of diabetes than those not on this medication (29.7% vs. 55.3%; $P = 0.019$). ADMA levels were higher among diabetics (0.38 ± 0.41 μmol/l) compared with nondiabetics (0.31 ± 0.25 μmol/l), but this was not statistically significant. However, in the diabetic patients subgroup, those who received paricalcitol had lower ADMA levels compared with those who did not receive paricalcitol with statistical differences ($P = 0.0342$). In the nondiabetic patients subgroup also, it was confirmed that those treated with paricalcitol had lower ADMA levels than those not treated with paricalcitol with a significant difference ($P = 0.0157$), as shown in Figure 3.

In addition, there was an inverse correlation between the weekly dose of paricalcitol and ADMA levels with a Pearson's and Spearman's correlation

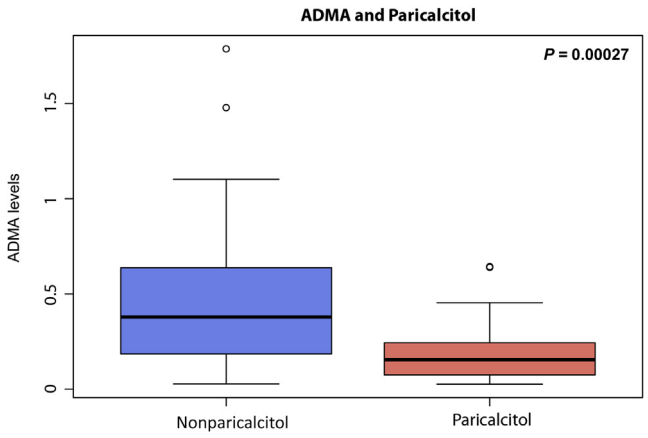

Figure 1. The difference between ADMA levels (μmol/l) in patients treated with paricalcitol compared with those who did not receive paricalcitol. ADMA, asymmetric dimethylarginine.

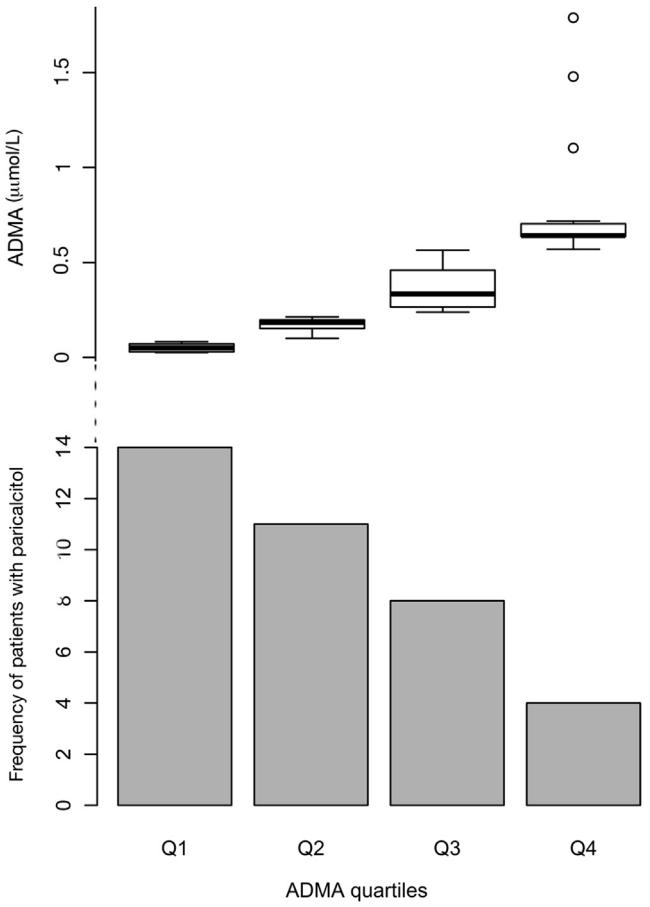

Figure 2. Quartile 1 shows that more patients were treated with paricalcitol and therefore they have lower levels of ADMA (μmol/l). Patients with higher levels of ADMA were less likely to receive treatment with paricalcitol ($P = 0.014$). ADMA, asymmetric dimethylarginine.

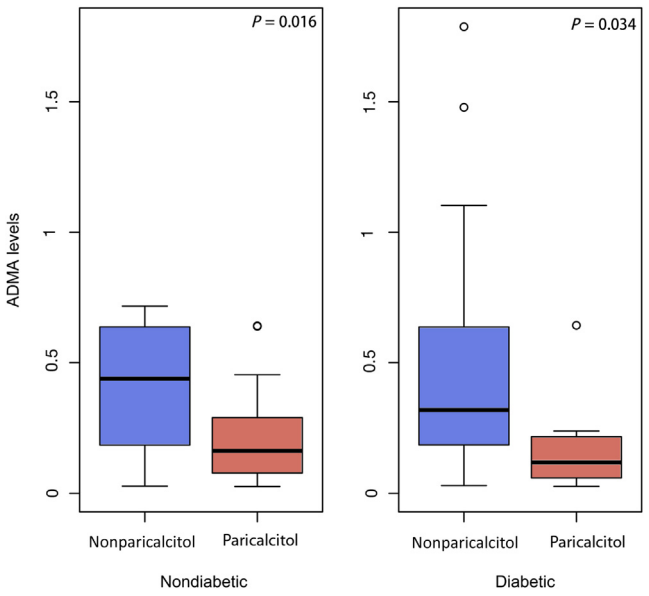

Figure 3. ADMA (μmol/l) levels of patients treated with paricalcitol and those not treated with paricalcitol in the nondiabetic and diabetic subgroups. ADMA, asymmetric dimethylarginine.

coefficient between paricalcitol and ADMA of −0.36 ($P = 0.013$; Figure 4) and −0.46 ($P = 0.001$), respectively. In the multivariable analysis, paricalcitol, age, and serum PTH levels were associated with ADMA levels; paricalcitol tended to show a significant association (Table 3).

At 36 months' follow-up, we observed an all-cause mortality of 30.1% with 28 deaths, 15 of which were due to cardiovascular events. In a model that only includes ADMA (as a continuous variable) as a predictor of mortality, its value is significant ($P = 0.0033$), but when other variables (age, sex, hypertension, and diabetes mellitus) are included in the model, ADMA looses significance as a mortality predictor (see Supplementary Figures S1–S3). We did not find the association between ADMA levels and cardiovascular cause mortality.

DISCUSSION

Hemodialysis patients on paricalcitol treatment had lower plasma ADMA levels according to our study. ADMA is significantly increased in ESRD.[5] Our study shows that dialysis patients treated with paricalcitol had significantly lower ADMA concentrations than those not treated with paricalcitol. ADMA, an endogenous methylated arginine analog, results from protein turnover, and its metabolism is facilitated by dimethylarginine dimethylaminohydrolase-1 and -2 isoforms. ADMA inhibits nitric oxide synthases, which may in part explain the impaired vasorelaxation, elevated inflammation, and reduced angiogenesis reported in patients and animal models of CKD.[21] Zoccali *et al.*[9] identified the plasma concentrations of ADMA as a predictor of mortality and cardiovascular disease in patients with chronic renal failure. On the other hand, data related to different medications that modify levels of ADMA have been reported.[13,14,19,22–24] Our study is the first, to our knowledge, to establish a link between ADMA and paricalcitol in hemodialysis patients.

Paricalcitol (19-nor-1,25-dihydroxyvitamin D2), a vitamin D analog with less hypercalcemic effect than calcitriol,[25] may provide a survival benefit for patients

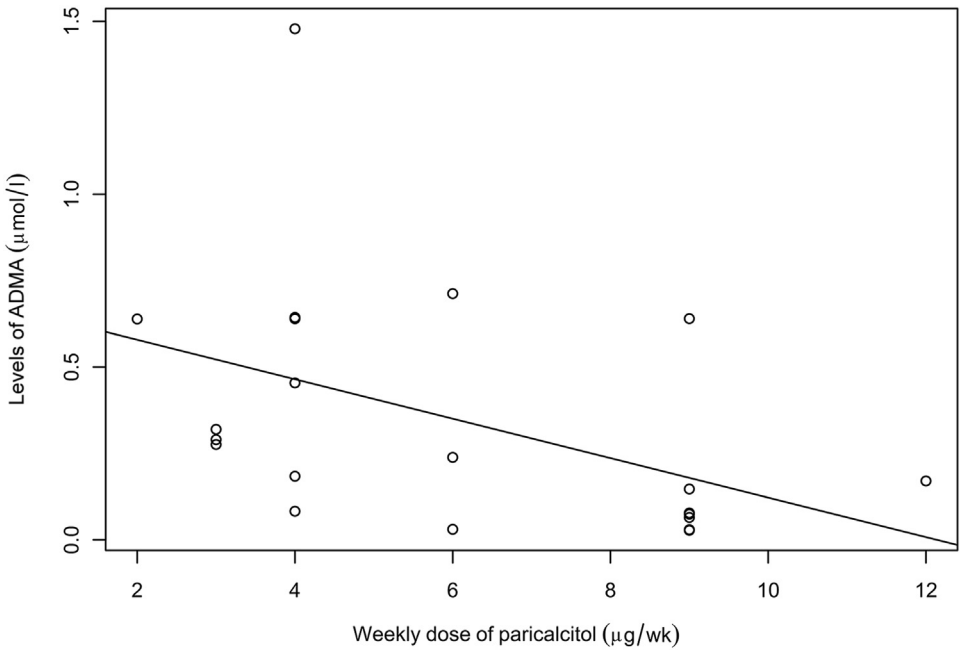

Figure 4. Inverse correlation between paricalcitol dose (mcg per week) and serum ADMA levels ($r = -0.36$, $P = 0.013$). ADMA, asymmetric dimethylarginine.

Table 3. Factors associated with ADMA levels: multivariate regression analysis using ADMA levels as a dependent variable

Independent variables	β-coefficient	SE	Beta (standardized)	95% CI	*P* value
Age (yr)	5.81	2.26	0.25	1.32, 10.31	0.012
Paricalcitol: yes	−183.52	60.56	−0.3	−303.93, −63.11	0.003
PTHi (ng/l)	0.16	0.07	0.21	0.01, 0.31	0.034

ADMA, asymmetric dimethylarginine; CI, confidence interval; PTH, parathyroid hormone.

with CKD, which is independent of the calcium and parathyroid hormone.[26,27] Paricalcitol could have a survival advantage in dialysis patients, possibly due to its ability to suppress PTH levels with less severe increments in serum calcium and phosphate.[28] Although paricalcitol reduces circulating bone turnover markers and PTH levels, the potential mechanism by which paricalcitol exerts its beneficial effect is not yet fully clarified. Recent studies report that this mechanism seems unrelated to fibroblast growth factor-23, as paricalcitol therapy increases the levels of fibroblast growth factor-23.[29] Paricalcitol has demonstrated effects on endothelial function and suppressing inflammation decreasing levels of high-sensitivity C-reactive protein, tumor necrosis factor-α, and interleukin-6 in hemodialysis patients,[12] but the effects of paricalcitol on ADMA require future studies.

Although our study was designed as a prospective study, confirming, like in other studies, that in our cohort ADMA levels predict overall mortality,[9,30–36] we mainly present cross-sectional results that we report as the main limitation. To conclude, we found that in hemodialysis patients, those treated with paricalcitol had lower levels of plasma ADMA concentrations. Future studies are needed to evaluate the relationship between ADMA and paricalcitol in ESRD and other stages of CKD. The effects paricalcitol can have on ADMA levels and the underlying endothelial function mechanisms still need to be studied.

DISCLOSURE

JCR-P received honoraria from Abbvie. All the other authors declared no competing interests.

ACKNOWLEDGMENTS

We would like to thank Dr. Rodriguez Perez, Dr. Marañes, and Dr. Payan for their great work and humanity. We would also like to thank Miguel Garcia-Bello and Jesus Maria Gonzalez-Martin for their work and effort.

SUPPLEMENTARY MATERIAL

Figure S1. Kaplan-Meier survival analysis evaluating the correlation between ADMA and all-cause mortality. Hazard ratio: 2.062; Confidence interval (CI) = [0.95; 4.47].

Figure S2. Relationship between ADMA (μmol/l) and medications used in dialysis patients.

Figure S3. Histogram showing ADMA levels (μmol/l) in patients treated with paricalcitol (red line) or not (black line). Patients treated with paricalcitol (in red) are grouped at the beginning of the graph, where ADMA levels are lower, whereas those patients not receiving paricalcitol (in black) are more evenly distributed along the vertical axis.

Supplementary material is linked to the online version of the paper at www.kireports.org.

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