Elevated PTH in AAs with CKD


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Contribution of calcium, phosphorus and 25-hydroxyvitamin D to the excessive severity of secondary hyperparathyroidism in African-Americans with CKD

Jennifer Ennis¹, Elaine Worcester² and Fredric Coe²

¹Litholink Corporation, Chicago, IL, USA and ²Section of Nephrology, Department of Medicine, University of Chicago, Chicago, IL, USA

Correspondence and offprint requests to: Jennifer Ennis; E-mail: ennisj1@labcorp.com

Abstract

Background. Parathyroid hormone (PTH) levels in African-American (AA) chronic kidney disease (CKD) patients exceed those in patients of other races; mechanisms are unknown.

Methods. We performed a cross-sectional analysis of initial laboratory data collected on 2028 CKD patients (505 AA) from US practices using a laboratory CKD service. Serum calcium (Ca), phosphorus (P), 25-hydroxyvitamin D (25-D) and plasma PTH levels were compared between the two groups.

Results. Mean PTH for AA exceeded PTH for non-AA in Stages 2–5 (P < 0.001, all four stages). 25-D levels were higher for non-AA in Stages 1–3 (P < 0.001). Serum Ca and P did not differ between groups at any stage. Full adjustment for these variables using multivariable generalized linear modeling did not remove the effect of AA race: AA PTH values exceeded non-AA values in CKD Stages 2–5 (P < 0.02, all four stages). Serum Ca, P and 25-D were all inversely correlated with PTH levels irrespective of race, but all factors combined accounted for ~42% of the variance in PTH.

Conclusions. PTH rises with progressive CKD stage far more in AA than in non-AA patients, and only a moderate component of the rise in PTH is explained by changes in serum Ca, P and 25-D in either group. These findings
concur with those from other large CKD cohorts and support the need for further study to determine other factors responsible for this racial difference.

**Keywords:** African-American; chronic kidney disease; mineral metabolism; parathyroid hormone

**Introduction**

It is well known that serum calcium (Ca) [1, 2], phosphorus (P) [1–4] and 25-hydroxyvitamin D (25-D) [5–8] influence parathyroid hormone (PTH) level in chronic kidney disease (CKD) and that changes in these three factors can account for some of the increase in PTH [1–8]. To this list, one can add 1, 25-dihydroxyvitamin D (1,25-D) [2, 9] and fibroblast growth factor 23 (FGF-23) [9–12] as well as age [13, 14], male sex [14], absence of diabetes [14–16] and African-American (AA) race [13, 14, 17]. The last of these seems to confer a selective worsening of secondary hyperparathyroidism as CKD progresses, but the reasons for this effect are unknown. To date, only three studies have specifically addressed the matter, and they vary among themselves in terms of population selection and range of variables considered [13, 14, 17].

Our focus here is to add evidence bearing on the high PTH of AA CKD patients derived from a wide variety of US private practices, both in primary care and in nephrology. Our hypothesis is that the effect of AA race on PTH, adjusted for several relevant clinical measures, is robust enough that it can be detected in such an unselected population. Our analysis, along with prior studies, leads to the conclusion that the racial effect is consistent, large and only partially explained by the usual measurements available in clinical practice.

**Materials and methods**

**Patients**

For this cross-sectional analysis, we studied 2028 patients drawn from 170 unscreened US primary care (112) and nephrology (58) practices making use of a convenient laboratory-based CKD service (Litholink Corporation, Chicago, IL, a subsidiary of LabCorp) from September 2008 through October 2010. As part of the service, the order form includes a query about demographic information, diabetic status and medication use. This information was provided by the practices to a variable extent. For this study, we selected only those patients for whom we could ascertain sex, age and race. We excluded patients known to be taking phosphate binders and active vitamin D analogs. In 58% of the cases studied, we could ascertain from the practices that patients were not taking either medication. In the remaining 42% of cases, medication use was unknown. For each patient, we selected the first complete set of laboratory data that contained creatinine for calculation of estimated glomerular filtration rate (eGFR), serum Ca, P and 25-D and plasma PTH all drawn on the same day. Only one set of laboratory results per patient was utilized for this analysis. PTH was analyzed using Roche reagent on a Roche Elecsys or COBAS E platform. 25-D was analyzed with the Diasorin Total D reagent on the Diasorin Liaison instrument. Serum Ca and P were analyzed with Roche reagent.

All laboratory data were ordered for clinical management and treatment decisions were at the discretion of the ordering clinician. eGFR calculations were made using the Modification of Diet in Renal Disease study equation and were corrected for AA race. CKD stage was assigned based upon the level of eGFR. Because clinicians chose to enroll patients in the Litholink CKD program, it was assumed that the diagnosis of CKD was made a priori by the clinician based upon available clinical data. No patient in Stage 5 received renal replacement therapy. Review exemption was granted from Western Institutional Review Board, Olympia, WA.

**Analysis**

We concerned ourselves with PTH, Ca, P, 25-D and eGFR. For each variable, we visually inspected the distribution of values using conventional probability plotting (p plot) and made use of standard tests for normality. Of the five variables, only Ca displayed reasonable normality, both within CKD stage and across all five CKD stages. Therefore, we log-transformed the remaining four variables and inspected them in the same manner. Although none of the four was strictly normal using conventional testing, their p plots were reasonably linear and therefore, we have used log-transformed values for this work. To make the results visually clear, we took the inverse logarithms and presented the results as standard numbers. Confidence limits were calculated from the log values and used to create 68% (1 SEM) intervals around mean values for plotting purposes. Comparisons of Ca, P, 25-D and PTH across CKD stages and between AA and non-AA patients within CKD stages were performed using Tukey’s significant difference test (Figure 1).

We performed stepwise multivariable regression analysis with PTH as the dependent variable and eGFR, Ca, P, AA group, the cross product of AA group with eGFR and 25-D as independent variables. We also inspected the individual effects of eGFR, Ca, 25-D and P by AA group, on PTH by creating four general linear models in which PTH was the dependent variable and CKD stage or pentiles of Ca, 25-D or P were categorical variables. For each model, the effect of the categorical variable was assessed adjusting for the other remaining continuous variables. AA group as well as the cross product of AA group with the categorical variable were included in the model so that comparisons of PTH between AA groups could be made for each pentile of the variable of interest. The values in Figure 2 represent the least square means by AA group: each model occupies one panel of the figure. Post hoc comparisons of PTH across pentile variables and between AA and non-AA patients were made within each model using Tukey’s significant different test (Figure 2). These analyses were performed using log-transformed data, except for Ca, and conventional software (SYSTAT Software, Inc., San Jose, CA).

**Results**

For both AA and non-AA groups (Table 1), patients in Stage 1 were younger than those in other stages. Mean age was higher in Stage 2 versus 1 and in Stage 3 versus 2. Prevalence of diabetes among AA patients exceeded non-AA patients: 37 versus 31% known diabetic, 34 versus 45% non-diabetic and 28 versus 23% not ascertained (χ² = 18, P < 0.001). As expected, PTH values were higher with increasing CKD stage (Figure 1, upper left panel); differences were significant (P < 0.001) for both groups between successive stages beginning at Stage 2. Values for AA exceeded non-AA in Stages 2–5 (P < 0.001, all four stages). Values exceeded the laboratory normal level (65 pg/mL) in Stages 4 and 5 for non-AA and 3–5 for AA. A supplemental analysis of variance was performed including only the relatively few (n = 1137) patients known to be not taking phosphate binders or active vitamin D. In that analysis (not shown), we could confirm significant differences between AA and non-AA PTH levels for Stages 2–4; Stage 5 had inadequate numbers for comparison.

Mean Ca (Figure 1, upper right panel) values were lower in Stage 4 compared to Stage 3 for non-AA (P = 0.045) and were lower in Stage 5 compared to Stage 4 for
AA (P = 0.017). All mean values were within the normal range for this laboratory (8.7–10.2 mg/dL).

Mean 25-D (Figure 1, lower left panel) values were below our laboratory normal range (32–100 ng/mL) for both groups in all stages. Values were higher in Stage 2 compared to Stage 1 for AA (P = 0.01) but were not different for either group between subsequent successive stages. Values for AA were significantly below those for non-AA in Stages 1–3 (P < 0.001).

Mean P (Figure 1, lower right panel) values were progressively higher across stages between Stages 3–5 (P < 0.05); values did not differ between groups within any stage. Mean values were within our laboratory normal range (2.5–4.5 mg/dL) except in Stage 5 for both groups.

**Multivariable analysis**

Because Ca, P, eGFR and 25-D affect one another through well-established physiological pathways, we performed multivariable regression analysis in order to determine their individual effects on PTH. In a stepwise analysis with PTH as dependent, eGFR was the leading covariate as judged by the F ratio (Table 2). The next variable to enter the model was 25-D, followed by AA group and Ca, whose F ratios were very similar. Diabetes, P and sex each entered the model but with very small effects on the $R^2$. Although the negative values for the standardized coefficients between PTH and Ca, eGFR and 25-D accord with physiological expectations, the signs of the standardized coefficients can be influenced by multiple correlations within the model and should not be interpreted mechanistically. The coefficient for the AA group was also negative, which was due to the interaction between AA and eGFR. Because there was a smaller percentage of AA patients at the later stages of CKD in this study and high PTH values correlate with later stages of CKD, being non-AA will correlate with higher PTH even though within a given stage, mean PTH values for AA exceeded those of non-AA. The significant cross product of AA and eGFR substantiates that AA status affects the interaction between PTH and eGFR, i.e. that within a given CKD stage, PTH will be higher in AA patients.

Compared to eGFR, the individual contributions of all other variables, as judged by the incremental increase of square multiple $R$ ($R^2$, Table 2), though significant, were of modest quantitative magnitude. The total $R^2$ for the model was 0.417, indicating that all these variables together accounted for ~42% of the variation in PTH in these patients. The high tolerance values for all variables except for the AA group and the cross product of the AA
group with eGFR indicate that each variable contributed in an independent manner. Age did not enter the model with significant effects.

Given these results, we inspected the individual effect of eGFR, Ca, 25-D and P, by AA group, on PTH adjusting for the other variables using four general linear models (Materials and methods). Mean PTH values adjusted for Ca, 25-D and P were higher with increasing CKD stage (Figure 2, upper left panel) for both AA and non-AA groups. Values for Stages 3–5 each exceeded the previous stage for both groups (P < 0.001); Stage 2 exceeded Stage 1 for AA. Adjusted values for AA exceeded non-AA for Stages 2 through 5 (P < 0.02, all comparisons). In Table 3, the F ratios further quantify the relative contribution of each variable within each of these models. In this model, the largest contributor was CKD stage (Table 3). Phosphorus had an extremely modest effect (F = 7), meaning it accounted for a tiny fraction of the variance. The steeper slope of PTH on CKD for AA versus non-AA (Figure 2, upper left panel) was significant based upon the F value of the cross product of AA group by CKD stage (Table 3).

For both AA and non-AA groups, PTH values were lower at higher Ca levels after full adjustment (Figure 2, upper right panel). For AA, PTH in the lowest Ca pentile exceeded that of all other pentiles, there was no progression of PTH change between subsequent pentiles. For non-AA, no two adjacent pentiles differed; however, any two non-adjacent pentiles differed significantly (P < 0.05). Adjusted PTH values for AA exceeded those for non-AA in all except the second pentile (P < 0.05). In this model, eGFR and 25-D had the greatest effects on PTH (Table 3).

For both AA and non-AA groups, PTH values were lower at higher 25-D levels after adjustment for eGFR, Ca and P (Figure 2, lower left panel). In both groups, PTH for the lowest pentile of 25-D exceeded values in the remaining pentiles. However, we did not detect lower PTH values with successive pentiles from the second through the fifth. Adjusted values for AA exceeded non-AA in all pentiles (P < 0.05). The largest covariate was eGFR (Table 3). 25-D had a detectable effect on PTH even after adjusting for the extremely large effects of eGFR, Ca and AA, but the effect arose mainly from the lowest 25-D pentile.

For both groups, we could detect a slight effect of P pentile on PTH (Table 3). However, there were no significant changes between pentiles of each group taken separately (Figure 2, lower right panel). In all pentiles, values for AA exceeded those of non-AA (P < 0.01, all
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Table 1. Patient characteristics

<table>
<thead>
<tr>
<th>CKD stage</th>
<th>Non-AA</th>
<th>AA</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>N (% female)</td>
<td>Age (years)</td>
<td>N (% female)</td>
<td>Age (years)</td>
</tr>
<tr>
<td>1</td>
<td>101 (41)</td>
<td>53 ± 1b</td>
<td>39 (67)</td>
</tr>
<tr>
<td>2</td>
<td>345 (51)</td>
<td>61 ± 1c</td>
<td>133 (56)</td>
</tr>
<tr>
<td>3</td>
<td>821 (54)</td>
<td>71 ± 1c</td>
<td>249 (61)</td>
</tr>
<tr>
<td>4</td>
<td>236 (55)</td>
<td>72 ± 1c</td>
<td>72 (67)</td>
</tr>
<tr>
<td>5</td>
<td>20 (45)</td>
<td>63 ± 3</td>
<td>12 (42)</td>
</tr>
<tr>
<td>Total</td>
<td>1523 (53)</td>
<td>67 ± 1</td>
<td>505 (61)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM; ages are men and women combined; SEM values are rounded to the nearest integer. Ethnicity is self-reported via physician offices. Non-AA: 94% White-Caucasian, 3% Hispanic, 2% Asian, 1% other.

Table 2. Determinants of PTH in CKD patients

<table>
<thead>
<tr>
<th></th>
<th>SC</th>
<th>Tolerance</th>
<th>F ratio</th>
<th>R²</th>
<th>ΔR²</th>
</tr>
</thead>
<tbody>
<tr>
<td>eGFR</td>
<td>−0.575</td>
<td>0.73</td>
<td>834</td>
<td>0.262</td>
<td>0.262</td>
</tr>
<tr>
<td>25-D</td>
<td>−0.230</td>
<td>0.94</td>
<td>172</td>
<td>0.333</td>
<td>0.071</td>
</tr>
<tr>
<td>AA group</td>
<td>−0.882</td>
<td>0.02</td>
<td>45</td>
<td>0.367</td>
<td>0.034</td>
</tr>
<tr>
<td>Calcium</td>
<td>−0.183</td>
<td>0.96</td>
<td>111</td>
<td>0.397</td>
<td>0.030</td>
</tr>
<tr>
<td>AA group × eGFR</td>
<td>0.707</td>
<td>0.02</td>
<td>29</td>
<td>0.405</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes</td>
<td>−0.062</td>
<td>0.93</td>
<td>12</td>
<td>0.409</td>
<td>0.004</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>−0.073</td>
<td>0.89</td>
<td>16</td>
<td>0.413</td>
<td>0.004</td>
</tr>
<tr>
<td>Sex</td>
<td>−0.055</td>
<td>0.94</td>
<td>10</td>
<td>0.416</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Results from stepwise multivariable regression model. Age did not enter the model. SC, standardized coefficient; tolerance is an estimate of independent effect; R² is the multivariable correlation coefficient; ΔR² is the increment of R² with addition of succeeding variables to the model.

Discussion

Our main result is that PTH levels are higher with increasing CKD stage and this phenomenon is more marked in AA than in non-AA patients. Only a moderate component of the effects of CKD stage and AA race is accounted for by alterations in serum Ca, 25-D and P. Of note, age had no effect on either the AA–non-AA difference or the effect of CKD on PTH. Sex and diabetes had detectable but extremely minor effects. However, we could ascertain diabetic status only in 72% of AA cases and in 77% of non-AA cases.

Ca metabolism in AA versus non-AA differs in subjects with normal renal function [18, 19]. In a study of subjects on a controlled low Ca (400 mg) diet, PTH and 1,25-D values of AA exceeded non-AA, while Ca and P did not differ [18]. In a study of healthy subjects from the National Health and Nutrition Examination Survey (NHANES), PTH was less responsive to 25-D in the range >20 mg/mL in Blacks compared to Whites [19]. Therefore, racial differences in CKD may well represent a pathological exaggeration of mechanisms that are present even in the normal condition.

Our work supplements prior studies of CKD patients by adding observations in Stages 1, 2 and 5. Specifically, our results agree with those of De Boer et al. [13], Gutierrez et al. [17] and Vassalotti et al. [14], except that De Boer et al. did not have significant Stage 2 data, Gutierrez et al. pooled Stages 1 and 2 and Stages 4 and 5 and Vassalotti et al. did not address Stages 1 or 2. In our study, we were able to find that PTH is significantly higher in AA by Stage 2 CKD and remains higher at subsequent stages. Like De Boer et al. [13], we found no differences in Ca by race, whereas Vassalotti et al. and Gutierrez et al. found higher Ca in some AA subjects [14, 17]. Like De Boer et al. and Vassalotti et al. [13, 14], we found no differences in P by race. Gutierrez et al. found that AA had higher P, but only in Stage 3b (eGFR 30–44 mL/min/1.73m²) [17]. Gutierrez et al. and we generally found lower 25-D values in AA patients [17]. Our differences reached significance only in Stages 1–3, but mean 25-D levels were below normal at all stages of CKD.

All four studies agree that PTH is higher in AA despite multivariable adjustments and that eGFR is a significant covariate [13, 14, 17]. Ca [13], P, 25-D [17], age [14], sex [14] and diabetes [14] were variably significant from study to study. Since the racial difference is not explained by differences in Ca, P, 25-D and eGFR, perhaps it may reflect effects of hormones such as FGF-23 and Klotho, which were not measured in this study. FGF-23 has been shown to increase progressively as early as Stage 2 CKD [11]. Gutierrez et al. [20] found that AA and non-AA patients had similar levels of FGF-23 and PTH both in health and in CKD, but AA patients had lower fractional excretions of phosphate, suggesting a racial difference in sensitivity to phosphaturic stimuli. A recent paper by Isakov et al. [21] suggests that higher values of FGF-23 may be found in AA CKD patients versus other races. By contrast, in dialysis patients, FGF-23 levels are significantly lower in AA than in non-Hispanic White patients [22].
and PTH levels are higher [23–26]. The role of FGF-23 in racial differences of PTH requires further study.

The effect of AA race on PTH is persuasive because these four studies analyzed different patient populations, yet came to the same conclusion. De Boer et al. [13] studied patients in one academic nephrology practice. The KEEP population (Vassalotti et al. [14]) arose from a national voluntary screening program. Gutierrez et al. [17] studied patients from the SEEK cohort which was drawn from community-based practices across the USA. Our population was also drawn from practices across the USA, relying only on the voluntary interest of particular physicians.

Our study has important limitations. Diet was unknown. No urine data were available and therefore, fractional reabsorptions and other indices of renal tubule function could not be gauged. We had medication information in only 58% of cases. We did not know socioeconomic status, body mass index (BMI) and smoking status, although smoking affects serum PTH values in healthy young women [27] and BMI correlates with PTH level in CKD patients and healthy women [14, 27]. Due to the cross-sectional nature of this study, patients may have been at various stages of treatment of CKD mineral bone disorder. Consequently, the ability to generalize about the natural history of CKD and its implications on mineral metabolism is limited. Despite this variability, which we would expect to obscure our findings, we are able to detect a racial effect on PTH.

Overall, CKD increases PTH more in AA than in non-AA patients. Our study is the first to show that this difference is apparent as early as Stage 2. Our study further suggests that neither the rise in PTH itself nor the difference between races can be fully accounted for by eGFR, Ca, P or 25-D. This matter is important because PTH is used as a gauge of CKD mineral bone disorder, a gauge that can drive therapeutic interventions. Almost certainly, the weight of evidence suggests that use of this gauge must be selective in relation to AA race, an issue that has not yet been addressed in any clinical guidelines.

Conflict of interest statement. J.E. is an employee of Litholink Corporation, a subsidiary of Laboratory Corporation of America (LabCorp) and hold stock in LabCorp. The research conducted was sponsored by this company. E.W. and F.C. are both consultants for Litholink Corporation and members of the Litholink CKD Scientific Advisory Board. The manuscript is not currently under review at any other journal. The results presented in this paper have not been published previously in whole or part, except in abstract form. All work for this project was undertaken at Litholink Corporation. All authors have made important contributions to the design and execution of this study, the analysis and interpretation of data and the writing of this manuscript.

(See related article by Isakova. Racial differences in parathyroid hormone levels in CKD. Nephrol Dial Transplant 2012; 27: 2616–2617.)

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**Urinary heparanase activity in patients with Type 1 and Type 2 diabetes**

Angelique L.W.M.M. Rops1,*, Mabel J. van den Hoven1,*, Bart A. Veldman1,2, Simone SALEmink1, Gerald Vervoort1,2, Lammy D. Elving2, Jan Aten3, Jack F. Wetzels1, Johan van der Vlag1 and Jo H.M. Berden1

1Nephrology Research Laboratory, Department of Nephrology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, 2Department of General Internal Medicine, Section Diabetes, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands and 3Department of Pathology, Amsterdam Medical Center, Amsterdam, The Netherlands

Correspondence and offprint requests to: Jo H.M. Berden; E-mail: J.Berden@nier.umcn.nl

*Both authors contributed equally to this study.

**Abstract**

**Background.** A reduced heparan sulphate (HS) expression in the glomerular basement membrane of patients with overt diabetic nephropathy is associated with an increased glomerular heparanase expression. We investigated the possible association of urinary heparanase activity with the development of proteinuria in patients with Type 1 diabetes (T1D), Type 2 diabetes (T2D), or membranous glomerulopathy (MGP) as non-diabetic disease controls.

**Methods.** Heparanase activity, albumin, HS and creatinine were measured in the urine of patients with T1D (n = 58) or T2D (n = 31), in patients with MGP (n = 52) and in healthy controls (n = 10). Heparanase messenger RNA (mRNA) expression in leukocytes was determined in a subgroup of patients with T1D (n = 19).

**Results.** Urinary heparanase activity was increased in patients with T1D and T2D, which was more prominent in patients with macroalbuminuria, whereas no activity could be detected in healthy controls. Albuminuria levels were associated with increased urinary heparanase activity in diabetic patients (r = 0.20; P < 0.05) but not in patients with MGP (r = 0.11; P = 0.43). A lower urinary heparanase activity was observed in diabetic patients treated with inhibitors of the renin–angiotensin–aldosterone system (RAAS), when compared to diabetic patients treated with other anti-hypertensives. Additionally, urinary heparanase activity was associated with age in T1D and MGP. In MGP, heparanase activity and β2-microglobulin excretion correlated. In patients with T1D, no differences in heparanase mRNA expression in leukocytes could be observed.

**Conclusions.** Urinary heparanase activity is increased in diabetic patients with proteinuria. However, whether increased heparanase activity is a cause or consequence of proteinuria requires additional research.

**Keywords:** membranous glomerulopathy; Type 1 diabetes; Type 2 diabetes; urinary heparanase activity

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**Introduction**

Diabetic nephropathy (DNP) is a frequent cause of chronic renal failure and end-stage renal disease. One of the first symptoms of DNP is an increased urinary excretion of albumin (30–300 mg/24 h) defined as microalbuminuria. Alterations in both size- and charge-selective properties of the glomerular filter have been implicated in