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Effects of diuretics furosemide and hydrochlorothiazide on CKD-MBD: A prospective randomized study

Raquel F.V. Vasco [a,*], Liliam Takayama [b], Rosa M.R. Pereira [b], Rosa M.A. Moyses [a], Rosilene M. Elias [a,c]

[a] Department of Medicine, Renal Division, Hospital das Clinicas HCFMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil
[b] Department of Medicine, Rheumatology Division, Hospital das Clinicas HCFMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil
[c] Universidade Nove de Julho, UNINOVE, São Paulo, Brazil

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ABSTRACT

Although diuretics are often prescribed to control fluid overload, they can change Chronic kidney disease-mineral and bone disorder (CKD-MBD) parameters. Previous studies have shown an association between diuretic prescription and changes in both calciuria and parathormone levels. However, the causal relationship could not be confirmed. In addition, the effects of diuretics on bone mineral density and turnover markers are yet to be established. To evaluate the effects of diuretics on CKD-MBD, we have performed a prospective randomized trial comparing hydrochlorothiazide with furosemide in a stage 3CKD population followed for 1 year. Furosemide increased bone remodeling and parathormone levels, whereas hydrochlorothiazide attenuated parathyroid hormone rise and decreased bone turnover markers.

1. Introduction

Loop diuretics and thiazide are often used for patients with CKD to control hypertension and hypervolemia. Most studies on diuretics have focused on effectiveness with impaired renal function and adverse events. However, diuretics may alter markers of mineral and bone metabolism in patients with CKD (CKD-MBD). Indeed, furosemide is capable of increasing calciuria and is associated with a high risk of fractures in long-term use (Rejnmark et al., 2006a). Opposite to in contrast, thiazide decreases calciuria, which is a desirable action for patients with nephrolithiasis, reducing the fracture risk (Rejnmark et al., 2005; Ptinopoulou et al., 2013).

Diuretics can potentially change levels of parathyroid hormone (PTH) (Zaheer et al., 2016) and other markers of CKD-MBD by altering the homeostasis of calcium (Rejnmark et al., 2006b; Rejnmark et al., 2003). Isakova et al., in a cross-sectional analysis, suggested that furosemide is associated with a higher risk for secondary hyperparathyroidism (Isakova et al., 2011). However, our group has previously demonstrated in a retrospective study that the effect of diuretics on PTH is not entirely explained by calcium homeostasis (Vasco et al., 2016). These results were hypothesis-generating and a prospective trial was still missing.

We designed a prospective randomized study testing the effect of a loop diuretic (furosemide – group FURO) and thiazide (hydrochlorothiazide – group HYDRO) to ascertain whether each diuretic may cause hyperparathyroidism and changes in bone markers in patients with CKD not on dialysis.

2. Materials and methods

This trial included adult patients with stage 3CKD in the period between August/2015 to January/2018. Patients signed consent and were treated for 1 year with Furosemide 40 mg/day or Hydrochlorothiazide 25 mg/day, after a washout period of 30 days. The study was blinded to the investigators, who prescribed drug A or B, but not to the participants, who received the respective prescription to each drug from a nurse coordinator. Exclusion criteria included diabetes, advanced cardiac and hepatic insufficiency, systolic blood pressure < 100 mmHg, previous or current use of bisphosphonate, steroid, calcium carbonate, or anticonvulsants drugs. Bone markers, PTH 1-84, intact FGF23, 1,25-hydroxyvitamin D, and Dual-Energy X-ray absorptiometry were assessed at baseline and 1 year. The Local Research Ethical Board has approved the

* Corresponding author at: Faculdade de Medicina da Universidade de São Paulo, Serviço de Nefrologia, Rua Dr. Enéas de Carvalho Aguiar 255, 7° andar, São Paulo CEP 05403-000, SP, Brazil.
E-mail address: raquelfvvasco@alumni.usp.br (R.F.V. Vasco).

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Table 1
Biochemical and bone mineral density parameters from baseline to 1 year, by study group.

	HYDRO			FURO					
	Baseline	6 months	1 year	Baseline	6 months	1 year	p Drug	p Time	P Interaction
Biochemistry									
eGFR	43.7 ± 9.0	42.3 ± 10.8	40.7 ± 9.9	45.0 ± 6.9	45.9 ± 8.4	41.7 ± 9.3	0.70	**0.02**	0.32
Total Ca (mg/dl)	9.6 ± 0.4	9.7 ± 0.4	9.7 ± 0.4	9.4 ± 0.4	9.5 ± 0.4	9.4 ± 0.6	0.08	0.79	0.81
Ionized Ca (mg/dl)	5.0 ± 0.2	5.1 ± 0.2	5.00 ± 0.17	5.0 ± 0.2	5.0 ± 0.2	4.99 ± 0.21	0.78	0.32	0.57
Urinary Ca (mg/24 h)	50.1 (20;92)	21.2 (12;58)	19.4 (14;45)	52.3 (31;130)	58 (41;109)	79 (35;105)	**0.009**	0.10	0.23
Excretion fraction Ca%	0.7 (0.4;1.3)	0.3 (0.2;0.7)	0.4 (0.2;0.7)	0.7 (0.4;1.5)	1.1 (0.6;1.3)	1.3 (0.9;1.8)	**0.001**	0.77	**0.01**
Phosphate (mg/dl)	3.3 ± 0.6	3.3 ± 0.5	3.3 ± 0.5	3.1 ± 0.6	3.2 ± 0.6	3.3 ± 0.7	0.77	0.77	0.82
Alkaline phosphatase (U/L)	70 (58;79)	62 (58;79)	65 (58;73)	69.5 (55;92)	74 (61;86)	77 (61.93)	0.24	0.88	0.31
Potassium (mEq/l)	4.7 ± 0.6	4.3 ± 0.5	4.2 ± 0.5	4.4 ± 0.2	4.4 ± 0.3	4.4 ± 0.4	0.76	**<0.001**	**0.012**
pH	7.35 ± 0.04	7.35 ± 0.05	7.35 ± 0.05	7.34 ± 0.04	7.36 ± 0.04	7.33 ± 0.04	0.7	0.33	0.34
Hormones and bone markers									
PTH (pg/ml)	71.2 ± 29.5	70.9 ± 26.9	79.2 ± 39.2	78.2 ± 23.4	75.6 ± 18.8	84.9 ± 29.3	0.50	0.19	0.97
PTH 1-84 (pg/ml)	38.1 ± 14.8	–	41.6 ± 16.6	38.6 ± 11.8	–	44.9 ± 14.4	0.65	**0.04**	0.57
25(OH)VitD (ng/ml)	36.2 ± 12.7	32.3 ± 7.3	35.1 ± 10.4	30.8 ± 7.6	33.1 ± 6.6	32.4 ± 8.2	0.45	0.55	0.07
1.25 $(OH)_2$VitD (pg/ml)	45.1 ± 13.3	–	38.4 ± 13.1	46.9 ± 14	–	50.0 ± 10.6	0.17	0.45	**0.046**
FGF 23 (pg/ml)	33 (14.3;49.6)	–	34.8 (22.5;34.8)	27.1 (21.1;36.6)	–	37.7 (20.7;58.7)	0.9	**0.03**	0.99
P1NP (ng/ml)	56.5 ± 29.6	–	50.5 ± 32.6	65.7 ± 68.6	–	81.2 ± 66.9	0.25	0.19	**0.005**
CTX (ng/ml)	0.39 ± 0.20	–	0.31 ± 0.24	0.40 ± 0.52	–	0.44 ± 0.46	0.59	0.23	**0.009**
BMD measurements									
Lumbar spine (g/cm^2)	1.091 ± 0.173	–	1.106 ± 0.174	1.000 ± 0.149	–	1.014 ± 0.152	0.45	**0.006**	0.87
T-score	−0.01 ± 1.64	–	0.06 ± 1.65	−0.85 ± 1.34	–	−0.76 ± 1.34	0.45	0.27	0.85
Total hip (g/cm^2)	0.972 ± 0.118	–	0.963 ± 0.117	0.918 ± 0.124	–	0.908 ± 0.127	0.52	**0.03**	0.92
T-score	−0.29 ± 0.74	–	−0.44 ± 0.71	−0.70 ± 0.79	–	−0.79 ± 0.83	0.82	**0.007**	0.65
Radius – ultradistal (g/cm^2)	0.458 ± 0.078	–	0.453 ± 0.069	0.471 ± 0.077	–	0.466 ± 0.082	0.18	**0.048**	0.82
Radius – upper 1/3 (g/cm^2)	0.734 ± 0.078	–	0.729 ± 0.074	0.766 ± 0.089	–	0.748 ± 0.081	0.51	**0.003**	0.08
Total body (g/cm^2)	1.108 ± 0.082	–	1.105 ± 0.091	1.098 ± 0.103	–	1.097 ± 0.109	0.52	0.72	0.52

Values are mean SD or median (25;75) unless indicated otherwise. p value from Linear Mixed Model with repeated measures, univariate analysis.
eGFR, estimated filtration rate; PTH, parathormone; Ca, calcium; 25(OH)VitD, 25-hydroxyvitamin D; 1,25 $(OH)_2$VitD, 1,25-dihydroxyvitamin D; FGF-23, fibroblast growth factor 23; P1NP, procollagen type I N-propeptide; CTX, Collagen type I C-telopeptide; BMD. bone mineral density.

protocol that was registered at Clinicaltrials.gov as NCT03082742.

We applied a linear mixed-effects model (LMM) to compare longitudinal changes on PTH, biochemical parameters, and bone markers across diuretic groups. All statistical tests were two-tailed, and the threshold of statistical significance was established at $p < 0.05$. We performed statistical analyses with SPSS version 21.0 (SPSS Inc., Chicago, IL). (Please see Supplementary methods for details).

3. Results

Forty patients were included in this study. Five patients were excluded due to the withdrawal of consent or symptomatic hypotension (Supplementary Fig. S1). The median age was 62, ranging from 43 to 79 years old, 65% were men, and 42.5% were white; hypertension was the main etiology of CKD (70%). Baseline clinical characteristics and biochemical parameters were similar between randomization groups; eGFR was 44 ± 8 ml/min/1.73m^2, PTH was 74.2 ± 25.6 pg/ml, phosphate and calcium were within the normal range, calciuria was 49.2 (21;91) mg/24 h (Supplementary Table S1).

After a 1-year follow-up, eGFR decreased ($p = 0.02$) from baseline with no difference between both groups ($p = 0.7$). The effect on calciuria was opposite, decreasing in HYDRO group and increasing in the FURO group ($p = 0.009$). PTH 1-84 and fibroblast growth factor 23 (FGF-23) increased in both groups. However, 1.25$(OH)_2$ Vitamin D, as well as procollagen type I N-propeptide (P1NP) and Collagen type I C-telopeptide (CTX), decreased in HYDRO group and increased in FURO group (Table 1).

Bone mineral density (BMD) results are presented in Table 1. In general, BMD has increased after 1 year at the lumbar spine (0.014 g/cm^2, $p = 0.006$), and decreased at the total hip (−0.010 g/cm^2, $p = 0.03$), ultradistal radius (−0.005 g/cm^2, $p = 0.048$), and upper 1/3 radius (−0.011 g/cm^2, $p = 0.003$). In the upper 1/3 radius, the loss in BMD seems to be more pronounced in the FURO group with a mean annual change of −2.3% (95% CI −4.0 to −0.7) in comparison to the HYDRO group with −0.6% (95% CI −1.9 to 0.8), $p = 0.08$. However, these results should be interpreted with caution as they did not reach the least significant change (LSC).

A linear mixed-effects model showed a longitudinal change in PTH 1-84, CTX and P1NP (Fig. 1A). There was a significant increase in PTH 1-84 in the FURO group (Fig. 1B), and a distinct change in CTX (Fig. 1C) and P1NP (Fig. 1D) according to each diuretic.

4. Discussion

In this RCT we demonstrated opposing effects of diuretics in CKD-MBD: loop diuretic caused an increase in PTH and bone turnover markers whereas thiazide attenuated the PTH rise and caused a reduction in bone turnover markers.

In a 1-year follow-up, we observed a slight reduction in eGFR, and therefore, one might expect an increase in PTH levels regardless of the diuretic use. However, the effect of furosemide on PTH was more pronounced, even adjusting for renal function. Therefore, we hypothesized that in the case eGFR remained constant, we would observe the opposite effects of the diuretics on PTH.

We challenged the current concept that changes in calciuria are the main responsible for changes in serum calcium and consequently in PTH levels. The mechanism by which calciuria stimulates PTH, however, is not totally revealed, and similarly to other authors (Reichel et al., 1992), we did not observe changes in serum calcium. Therefore, alternative mechanisms might have contributed to the effect on PTH such as the direct effect of furosemide on the parathyroid gland, through NKCC1 or calcium receptor, which was demonstrated in a randomized clinical trial that included 13 healthy men that received either cinacalcet or placebo (Muller et al., 2015). The authors demonstrated that furosemide increases PTH levels without any change in serum calcium, a response blunted by the previous administration of cinacalcet. In addition, it has

A

Variable	PTH 1-84 p value	CTX p value	P1NP p value
Diuretic type	0.03	0.59	0.45
Time	0.45	0.12	0.64
Diuretic type*Time	0.40	0.01	<0.001
Diuretic type*eGFR	0.06	0.72	0.43
Covariates			
eGFR	0.01	0.11	0.07
Age	0.98	0.98	0.47
Gender	0.94	0.47	0.67
1,25-dihydroxyvitamin D	0.13	0.64	0.17
Total Calcium	0.49	-	-
FGF 23	0.15	-	-

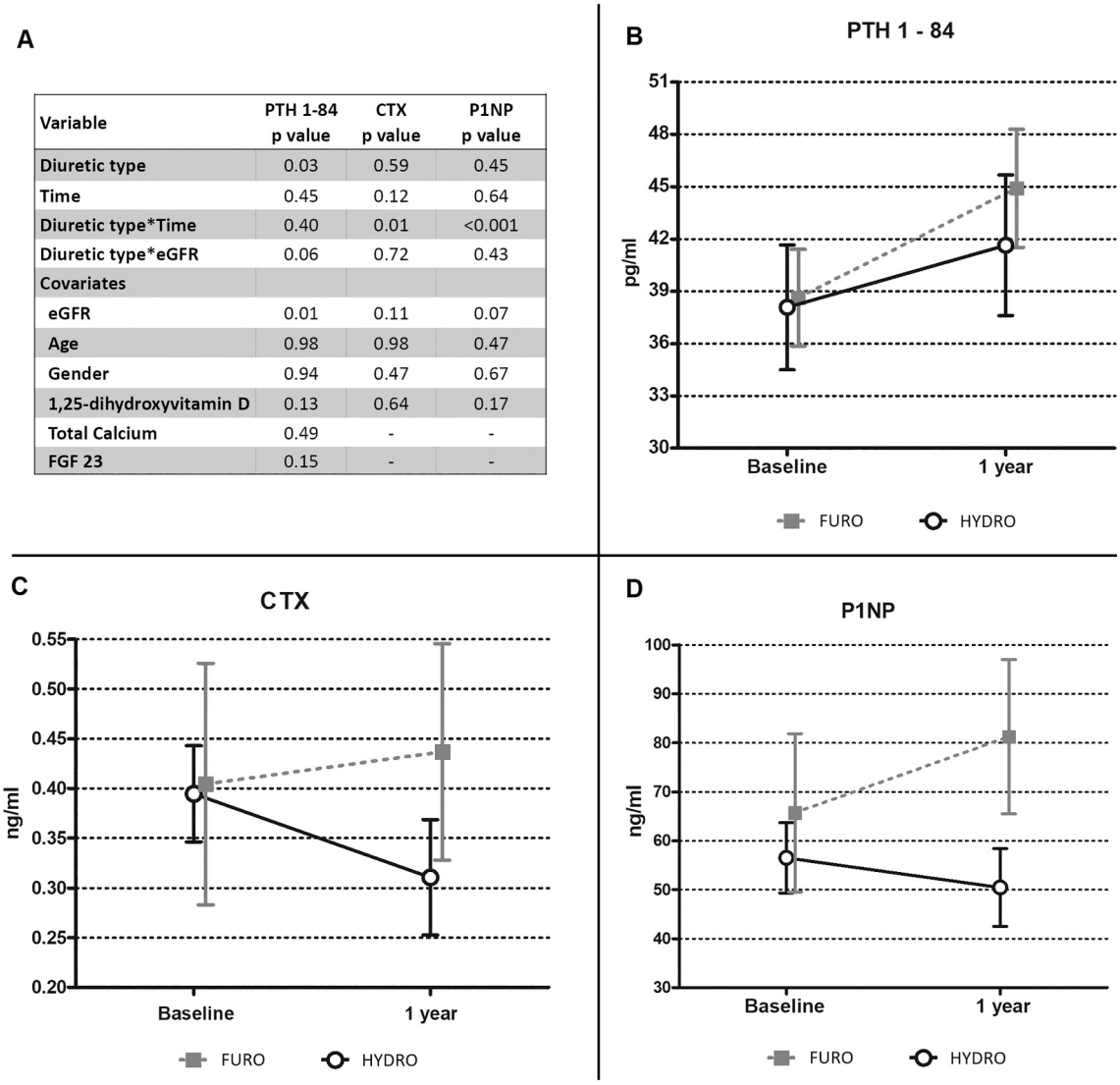

Fig. 1. Effect of diuretic type on PTH 1-84 and bone biomarkers.
A. Multivariate analysis of diuretic effect on PTH1-84, CTX and P1NP. p values are from repeated-measures Linear Mixed Model, covariates evaluated by AR (1) heterogeneous.
B. Longitudinal variation (mean ± SEM) in PTH 1-84 by study group.
C. Longitudinal variation (mean ± SEM) in CTX by study group.
D. Longitudinal variation (mean ± SEM) in P1NP by study group.
PTH, parathyroid hormone; CTX, C terminal telopeptide of type I collagen; P1NP, procollagen of type-1 N-terminal propeptide; eGFR, estimated glomerular filtration rate according to CKD-EPI equation; FGF 23, fibroblast growth factor; FURO furosemide group; HYDRO, hydrochlorothiazide group.

been described as a thiazide action increasing intestinal calcium absorption and osteoblast differentiation, in an experimental study with inactivation of thiazide-sensitive Na—Cl cotransporter gene (Hsu et al., 2015). Taken together, the data obtained from our subjects strongly suggest a complex mechanism explaining the changes in PTH levels caused by diuretics.

The effect of diuretic on PTH has previously been shown on bone turnover markers. Previous studies have demonstrated that bone turnover markers were lower in hypertensive women using thiazide when compared to other antihypertensive agents (Olmos et al., 2010) and a loop diuretic (bumetanide) was associated with an increase in levels of bone turnover markers after 1 year (Rejnmark et al., 2006b). For the first time, our study shows the effect of diuretics on bone remodeling in a CKD population. FGF-23 increased in both groups, which seems to be related to the decrease in renal function. Interestingly, we observed that furosemide increased whereas thiazide decreased the levels of 1,25 $(OH)_2$ vitamin D. This result suggests that each diuretic has a unique effect on the activation of vitamin D, although the mechanisms are unknown.

In a population with normal renal function, loop diuretics were associated with a loss in BMD at the total hip, ultradistal radius, and whole body (Rejnmark et al., 2006b), while hydrochlorothiazide reduced cortical bone loss after 2 years of follow-up (Reid et al., 2000). Nickolas et al. have demonstrated a cortical bone loss in patients with CKD and hyperparathyroidism, in association with elevated bone turnover (Nickolas et al., 2013). In our study, the use of thiazide seems to attenuate this loss. However, this result might be biased due to the small sample size.

We acknowledge there are some limitations to our study: it is a one center study, our findings could not be generalized to advanced CKD, and our power calculations did not include BMD outcomes. Finally, we have not tested a higher dose of thiazide and the dose-effect curve was not addressed. Despite these limitations, and in light of the paucity of compelling data currently available, this is the first RCT to demonstrate

an independent effect of diuretics on PTH and bone markers in a CKD population. We emphasized the complex nature of urinary and serum calcium and changes in PTH, highlighting the need for additional studies with longer follow up to incorporate this knowledge in daily clinical practice.

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Transparency document

The Transparency document associated with this article can be found, in online version.

CRediT authorship contribution statement

Raquel F.V. Vasco: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization. **Liliam Takayama:** Investigation, Data curation. **Rosa M.R. Pereira:** Resources, Writing – review & editing. **Rosa M.A. Moyses:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision. **Rosilene M. Elias:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision.

Declaration of competing interest

RFVV and LT: nothing to declare. Financial support: RMRP, RMAM and RME are supported by CNPQ, Conselho Nacional de Desenvolvimento Científico e Tecnológico (grant numbers 305556/2017-7, 304249/2013-0 and 305106/2018-0, respectively). This financial support had no role in study design; collection, analysis, and interpretation of data; writing the report; and the decision to submit the report for publication.

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