THE PREVALENCE OF BIOCHEMICAL ABNORMALITIES OF CHRONIC KIDNEY DISEASE. MINERAL AND BONE DISORDERS IN UNTREATED NON-DIALYSIS PATIENTS – A MULTICENTER STUDY

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Abstract

Background. There are scarce data about prevalence of mineral metabolism (MM) disorders in Romanian predialysis patients, so we assessed their occurrence and relationships in mild to severe chronic kidney disease (CKD).

Methods. One hundred fifteen non-dialysis CKD (eGFR 31, 95% CI 29-35mL/min) and 33 matched non-CKD subjects entered this multicentric, cross-sectional study. Serum 25-hydroxyvitamin D (25OHD), intact parathyroid hormone (iPTH), phosphate (PO₄), total calcium (tCa) and alkaline phosphatase (AP) were measured, along with demographic and past medical history data.

Results. Hypovitaminosis D was equally prevalent in Controls and CKD (91% vs. 96% had 25OHD<30ng/mL). Increasing proportions of hyperparathyroidism (33% - stage 2 to 100% - stage 5; p<0.001) and hyperphosphatemia (2% - stage 3 to 38% - stage 5; p<0.001) were found. Hypocalcemia was more prevalent in stage 5 (25% vs. 6% in stage 4, none in stage 3 and Controls, p<0.001). Mineral metabolism parameters correlated with eGFR. In addition, iPTH was directly associated with PO₄, AP, and urinary albumin-to-creatinine ratio (ACR), but inversely with tCa and 25OHD, while negative correlation of 25OHD with age, AP, ACR, and C-reactive protein emerged. In multiple regression, eGFR was the only predictor of iPTH (Beta -0.68, 95%CI -1.35 to -0.90, R² 0.46, p<0.001), whereas age and ACR were the determinants of 25OHD (a model which explained 14% of its variation).

Conclusions. Hypovitaminosis D was very common irrespective of CKD presence and severity, and it seems worsened by older age and higher albuminuria. Hyperparathyroidism preceded hyperphosphatemia and hypocalcemia, and it seems mostly dependent on kidney function decline.

Key words: Chronic kidney disease, vitamin D deficiency, secondary hyperparathyroidism, hyperphosphatemia, hypocalcemia.

INTRODUCTION

Disorders of the mineral metabolism are common complications of chronic kidney disease (CKD). Serum vitamin D, parathyroid hormone, phosphate and calcium abnormalities are part of a broad spectrum of disorders that occur in this clinical setting and result in both skeletal and extraskelatal consequences, named CKD-related mineral and bone disorder (CKD-MBD) (1, 2). Skeletal disturbances have traditionally been termed renal osteodystrophy and classified on the basis of bone biopsy. Together with ectopic (mainly arterial) calcifications they exert a noticeable impact on patients' morbidity and mortality (2, 3). Disorders of mineral metabolism were associated with all-cause, cardiovascular, infection-related, fracture-related, and vascular access-related hospitalization, as well as mortality in dialysis and non-dialysis CKD patients (4-6).

The processes causing disordered mineral metabolism and bone disease have their onset in the early stages of CKD, continue throughout the course of progressive loss of kidney function, and may be influenced, beneficially or adversely, by various therapeutic approaches (2). Information about the prevalence of biochemical abnormalities of CKD-MBD is important because it represents the first step towards planning and enacting adequate therapeutic measures to prevent and control CKD-MBD derived morbidities. Despite the known early occurrence, the great majority of studies on CKD-MBD and the prevalence of its components were conducted on dialysis patients. However, obtaining data from patients who are not receiving renal replacement therapy yet would be even more valuable, since any therapeutic intervention

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that is initiated early enough could have a better rate of success. Moreover, the prevalence of calcium-phosphate metabolism abnormalities, especially but not limited to vitamin D deficiency, could vary according to geographical region and economical status, so the prevalence reported in the USA or Japan does not necessarily correspond to the situation encountered in Romania, a country with temperate continental climate in which fish consumption is not very popular.

Consequently, the current study aimed to assess the prevalence and relationships of the biochemical abnormalities of mineral metabolism in non-dialysis CKD patients from two major nephrological departments which provide specialty care for the south and south-west of Romania.

SUBJECTS AND METHODS

This multicentric, cross-sectional, non-interventional study enrolled over a ten months period, 115 adults with known CKD and 33 matched non-CKD subjects, selected from the newly admitted patients according to the inclusion and exclusion criteria. The study was conducted in accordance with the Helsinki Declaration, with subsequent amendments. Every eligible patient was provided with detailed explanations about the goal and the plan of the research, and a signed informed consent form was obtained before any study procedure.

Subjects

The subjects were selected from one of the two participating tertiary care centres, provided they were older than 18 years and have a diagnosis of CKD, identified and staged according to the KDIGO criteria: estimated glomerular filtration rate (eGFR) $<90 \text{ mL/min/1.73m}^2$ (by abbreviated Modification of Diet in Renal Disease equation - MDRD) along with urinary albumin-to-creatinine ratio $>30\text{mg/g}$, both persistent for more than 3 months (7). Patients with autosomal dominant polycystic kidney disease, diagnosed by Ravine criteria (8), were included even if urinary albumin excretion was normal. Exclusion criteria were: renal replacement therapy (dialysis and renal transplantation), acute kidney injury, nephrotic proteinuria ($>3\text{g/g}$ urinary creatinine), active infectious or inflammatory diseases, malignancies, active liver diseases, malabsorption-maldigestion syndromes, treatments (current or in the past six months) with immunosuppressive drugs, bisphosphonates, vitamin D receptor activators, intestinal phosphate binders, and

parathyroidectomy.

The control group consisted of 33 adults, in stable clinical condition, without CKD, as demonstrated by a normal urinalysis (no proteinuria, cylindruria, hematuria or leukocyturia) and kidney ultrasound in association with $\text{eGFR} >60\text{mL/min/1.73m}^2$. These subjects were also selected from among the patients attending the two Nephrology Departments, during the same time period.

All subjects were of Caucasian origin. Half were aged >60 years, predominantly male (56%), with high prevalence of arterial hypertension (76%), clinical manifest atherosclerotic disease (47%), but relatively low prevalence of diabetes mellitus (23%), active smoking (14%) and declared alcohol consumption (12%). None were treated with calcium salts or vitamin D derivatives. The etiology of CKD was dominated by vascular nephropathies (40%), followed by chronic glomerulonephritis (21%), chronic tubulointerstitial nephritis (21%), hereditary nephropathies (9%), diabetic nephropathy (8%) and other causes.

Methods

Demographic information and complete medical history (lifestyle and dietary habits, kidney disease, co-morbidities, chronic medications) were obtained by medical interview. Body mass index (BMI) and arterial blood pressure (BP) were recorded after physical examination. Fasting blood and first morning void urine samples were collected for the assessment of mineral metabolism and routine biochemistry parameters. The samples were locally processed within one hour after collection and were transported (either on dry ice or at ambient temperature) on the day of collection to the same central laboratory, where all the measurements were performed.

Calcemia, phosphatemia, alkaline phosphatase, and all the other routine biochemical variables (including urinary concentrations of albumin, total protein and creatinine) were measured by standard spectrophotometric methods on multiparameter autoanalyzer (BS300, Mindray, China). Laboratory reference range values for total calcemia (tCa) were $8.5\text{--}10.5\text{mg/dL}$, for phosphatemia (PO_4) $2.0\text{--}5.0\text{mg/dL}$, and for alkaline phosphatase (AP) $42\text{--}140\text{UI/L}$. Serum intact parathyroid hormone (iPTH) was measured by an electrochemiluminescent test with 2 polyclonal, "sandwich-like" antibodies in two stages, with two binding sites (N-TACT®, Liaison DiaSorin). The limits of detection are $1\text{--}2000\text{pg/mL}$ and the variation coefficients for repetition (intra-determination) and

reproduction (inter-determination) are 4.8% and 5.9%, respectively (as reported by the manufacturer). The normal reference interval of this method is between 17.2 and 72.5pg/mL. An automated direct competitive immunologic technique, based on chemiluminescence (CLIA Liaison® DiaSorin), was used for determination of serum total 25-hydroxy-vitamin D (25OHD). The limits of detection are 4-150ng/mL and the normal reference values are 30-100ng/mL. The producer reported 2.9-5.5% intra-determination and 7.9-12.7% inter-determination variation coefficients of this method.

In accordance with KDIGO guidelines, values above or below the laboratory reference range were considered abnormal for iPTH, tCa and PO₄ regardless of the CKD stage (2). Vitamin D deficiency and insufficiency were defined at values of 25OHD <10ng/mL and between 10-30ng/mL, respectively (2).

Statistical analysis

Data are presented as mean ± standard deviation (SD), median with 95%CI, or percentages and were compared by ANOVA, Mann-Whitney and Chi2 tests, as appropriate according to their type and

distribution (verified by Kolmogorov-Smirnov test). Bivariate (Spearman rs correlation coefficient) and multiple linear regression analyses (on logarithmic transformed variables in order to correct the skewed distribution) were performed to assess correlations. A p value <0.05 was considered significant.

Microsoft Excel® and SPSS software packages were used.

RESULTS

No differences in demographic, lifestyle and medical history data were seen between the studied groups except for male gender, arterial hypertension and cardiovascular diseases, which were more common in CKD subjects (Table 1). In addition, angiotensin antagonist drugs were more frequently prescribed in this group. As expected, CKD patients had lower eGFR and higher albuminuria, as well as elevated mean arterial BP. Also, serum hemoglobin and albumin were lower, while C-reactive protein levels (as marker of inflammation) were higher in this group. CKD patients had higher serum iPTH and phosphate, although the other studied parameters of the mineral metabolism were similar in

Table 1. Main characteristics of the studied groups

Parameter*	non-CKD group (N=33)	All CKD group (N=115)	p
Age (years)	59 (49 to 60)	61 (56 to 62)	0.15
Gender (female, %)	61	39	0.03
Post-menopausal women (%)	39	27	0.17
Active smoking (%)	18	12	0.55
Alcohol consumption (%)	12	10	0.66
Meat consumption (days/wk.)	3 (3.1 to 4.6)	3 (3.0 to 3.0)	0.26
Fish consumption (days/wk.)	1 (0.9 to 1.6)	1 (1.1 to 1.5)	0.68
Diary consumption (days/wk.)	5 (3.2 to 5.3)	3 (2.8 to 3.8)	0.10
Cheese consumption (days/wk.)	6 (3.9 to 5.6)	4 (4.0 to 4.9)	0.45
History of fractures (%)	15	23	0.35
Diabetes mellitus (%)	18	24	0.46
Cardiovascular diseases (%)	24	54	0.002
Arterial hypertension (%)	54	82	0.001
ACEI/ARB treatment (%)	30	55	0.01
Mean arterial BP (mmHg)	90 (88 to 97)	97 (95 to 101)	0.04
Body mass index (kg/m ²)	26.7 (24.8 to 28.7)	27.0 (26.7 to 28.7)	0.41
Serum hemoglobin (g/dL)	13.9 ± 0.8	12.5 ± 1.2	0.001
Serum albumin (g/dL)	4.6 ± 0.3	4.4 ± 0.4	0.01
C-reactive protein (mg/L)	4 (5 to 8)	2 (2 to 5)	0.01
Estimated GFR (mL/min)	71 (66 to 77)	31 (29 to 35)	<0.001
Urinary ACR (mg/g)	8.6 (6.7 to 10.5)	182 (459 to 750)	<0.001
Serum iPTH (pg/mL)	56 (49 to 66)	97 (130 to 190)	<0.001
Serum 25OHD (ng/mL)	13.8 (13.2 to 20.1)	13.0 (12.7 to 15.3)	0.28
Serum total calcium (mg/dL)	9.4 (9.4 to 9.8)	9.5 (9.3 to 9.6)	0.63
Serum phosphate (mg/dL)	3.2 (3.1 to 3.4)	3.7 (3.6 to 4.0)	0.003
Serum total AP (UI/L)	71 (70 to 85)	84 (82 to 95)	0.15

* Expressed as median (95%CI), mean ± standard deviation, or percentage; CKD: chronic kidney disease; wk.: week; ACEI: angiotensin converting enzyme inhibitors; ARB: angiotensin receptor blockers; BP: blood pressure; GFR: glomerular filtration rate; ACR: albumin-to-creatinine ratio; iPTH: intact parathyroid hormone; 25OHD: calcidiol; AP: alkaline phosphatase

both groups (Table 1).

A high prevalence of vitamin D insufficiency (25OHD =10-30ng/mL) and deficiency (25OHD <10ng/mL) was equally found in both groups (64% and 27% in Controls, *versus* 65% and 32% in CKD group, respectively; $p > 0.05$) (Fig. 1) and in all CKD stages (Table 2). Conversely, there was a gradual increase in prevalence of hyperparathyroidism (HPTH, defined by serum iPTH >73 pg/mL) across CKD classes, from around one third of patients in stages 2, to all subjects in stage 5 CKD. The prevalence of hyperphosphatemia ($\text{PO}_4 > 5\text{mg/dL}$) increased as the eGFR declined below 30mL/min/1.73 m² (in stage 4 and 5 CKD), while hypocalcemia (tCa <8.5mg/dL) was more prevalent only in stage 5 CKD (Table 2, Fig. 2).

Elevated serum alkaline phosphatase (>140 IU/L) was overall present in one out of five CKD patients and its prevalence showed a trend to increase with CKD stage, but reached statistical significance *versus* controls only in stage 5 patients (44 *vs.* 9%, $p=0.01$), probably as a consequence of the secondary hyperparathyroidism-related increase in bone activity during advanced CKD.

In CKD patients, calcidiol levels and total serum calcium were weakly directly correlated with the eGFR ($r_s=0.19$, $p=0.02$ and $r_s=0.18$, $p=0.03$, respectively), while stronger inverse relations were observed in case of serum phosphate ($r_s=-0.59$, $p<0.001$), alkaline phosphatase ($r_s=-0.27$, $p=0.001$), and iPTH (Fig. 3). Additionally, serum parathyroid hormone was directly associated with PO_4 , AP and urinary albumin-to-creatinine ratio (ACR), and inversely with tCa and 25OHD, but had no correlation with age, CKD vintage, body mass index, serum albumin, or C-reactive protein (Table 3). However, in a model of multivariate regression analysis, which explained 46% of iPTH variation, eGFR was the only independent determinant of PTH level (Table 4).

As concerns the serum calcidiol (Fig. 4), although significant correlations were found with C-reactive protein, age, ACR, and AP, besides the already mentioned relations with eGFR and iPTH (Table 3), only older age and greater albuminuria emerged as independent determinants of the hypovitaminosis D in multivariate analysis (Table 5).

Table 2. The main studied parameters by chronic kidney disease stages

Parameter*	Stage 2 CKD (n=9)	Stage 3 CKD (n=56)	Stage 4 CKD (n=34)	Stage 5 CKD (n=16)	<i>p</i>
Age (years)	49 (43 to 62)	62 (59 to 67)	57 (50 to 63)	60 (51 to 66)	0.07
Gender (male, %)	67	61	66	75	0.13
Cardiovascular disease (%)	22	59	50	62	0.17
Diabetes mellitus (%)	0	23	27	23	0.21
History of fractures (%)	44	25	15	12	0.19
CKD vintage (years)	1.0 (0.1 to 6.0)	3.0 (3.4 to 5.4)	5.0 (3.8 to 9.6)	5.0 (3.4 to 6.7)	0.10
Serum albumin (g/L)	4.4 $\pm$ 0.6	4.5 $\pm$ 0.4	4.4 $\pm$ 0.4	4.2 $\pm$ 0.5	0.16
C-reactive protein (mg/L)	3 (1 to 4)	4 (4 to 9)	4 (4 to 9)	5 (4 to 16)	0.22
Urinary ACR (mg/g)	32 (-231 to 693)	46 (164 to 406)	478 (545 to 1155)	1290 (934 to 1887)	<0.001
Serum iPTH (pg/mL)	48 (38 to 81)	72 (71 to 98)	165 (148 to 249)	416 (285 to 518)	<0.001
iPTH >73pg/mL (%)	33	48	74	100	<0.001
Serum 25OHD (ng/mL)	19.4 (13.9 to 23.5)	13.4 (12.5 to 16.8)	12.5 (10.9 to 14.6)	11.9 (9.1 to 14.9)	0.11
25OHD <30ng/mL (%)	89	95	100	100	0.85
Serum total calcium (mg/dL)	9.9 (9.3 to 10.2)	9.5 (9.4 to 9.7)	9.6 (9.2 to 9.7)	9.3 (8.3 to 9.7)	0.29
tCa <8.5mg/dL (%)	0	0	6	25	0.09
Serum phosphate (mg/dL)	3.1 (2.9 to 3.8)	3.3 (3.1 to 3.5)	4.0 (3.9 to 4.5)	4.6 (4.2 to 5.8)	<0.001
$\text{PO}_4 > 5.0\text{mg/dL}$ (%)	0	2	18	38	<0.001
Serum total AP (U/L)	67 (51 to 82)	76 (76 to 94)	95 (79 to 102)	107 (85 to 127)	0.03
AP >140U/L	0	14	24	44	0.03
One biochemical CKD-MBD abnormality (%) [#]	33	48	76	100	<0.001
Two biochemical CKD-MBD abnormalities (%) [#]	0	2	18	38	<0.001
Three biochemical CKD-MBD abnormalities (%) [#]	0	0	3	25	0.06

* Expressed as median (95%CI), mean $\pm$ standard deviation, or percentage; #: The simultaneous presence of one, two or three abnormal values of the main biochemical mineral metabolism parameters (i.e. iPTH>73pg/mL and/or $\text{PO}_4 > 5.0\text{mg/dL}$ and/or tCa<8.5mg/dL); CKD: chronic kidney disease; ACR: albumin-to-creatinine ratio; iPTH: intact parathyroid hormone; 25OHD: calcidiol; tCa: total calcium; PO_4 : phosphate; AP: alkaline phosphatase; CKD-MBD: CKD-related mineral and bone disorder.

Figure 1. Prevalence of biochemical mineral metabolism abnormalities in CKD and non-CKD groups. CKD: chronic kidney disease; vit. D: 25-hydroxy-vitamin D (calcidiol); Low vit. D: serum calcidiol <30ng/mL; HPTH: hyperparathyroidism (defined as serum intact parathyroid hormone >73pg/mL); PO₄: serum phosphate; HyperPO₄: PO₄ >5.0mg/dL; Ca: serum total calcium; HypoCa: Ca <8.5mg/dL; AP: serum total alkaline phosphatase; High AP: AP >140IU/L.

Figure 2. Prevalence of hyperparathyroidism, hyperphosphatemia and increased alkaline phosphatase across CKD stages. CKD: chronic kidney disease; HPTH: hyperparathyroidism (defined as serum intact parathyroid hormone >73pg/mL); PO₄: serum phosphate; HyperPO₄: PO₄ >5.0mg/dL; AP: serum total alkaline phosphatase; High AP: AP >140IU/L.

Table 3. Bivariate correlations of serum parathyroid hormone and calcidiol with other studied parameters in chronic kidney disease group (N=115)

Parameter	iPTH		25OHD	
	rs	p	rs	p
Age	0.01	0.90	-0.31	0.001
eGFR	-0.66	<0.001	0.19	0.02
Urinary albumin-to-creatinine ratio	0.43	<0.001	-0.31	0.001
C-reactive protein	0.14	0.13	-0.34	<0.001
Serum total calcium	-0.23	0.01	-0.01	0.87
Serum phosphate	0.43	<0.001	-0.18	0.05
Alkaline phosphatase	0.26	0.005	-0.22	0.02
25OHD	-0.19	0.04	-	-
iPTH	-	-	-0.19	0.04

eGFR: estimated glomerular filtration rate; iPTH: intact parathyroid hormone; 25OHD: calcidiol; rs: Spearman rank correlation coefficient.

Table 4. Predictors of serum parathyroid hormone increase in chronic kidney disease patients

Predictor	Beta	95% CI	p
Intercept	-	7.68 to 9.20	<0.001
Ln(eGFR)	-0.68	-1.35 to -0.90	<0.001

Dependent variable: Ln(iPTH). Independent variables included in the first step: Ln(eGFR), Ln(PO₄), Ln(tCa), Ln(25OHD), Ln(AP), Ln(ACR). Adjusted R² = 0.461, p<0.001. N=115. 95% CI: 95% confidence interval; iPTH: serum intact parathyroid hormone; eGFR: estimated glomerular filtration rate; PO₄: serum phosphate; tCa: serum total calcium; 25OHD: serum 25-hydroxy-vitamin D; AP: serum total alkaline phosphatase; ACR: urinary albumin-to-creatinine-ratio

Table 5. Predictors of low levels of calcidiol in chronic kidney disease patients

Predictor	Beta	95% CI	p
Intercept	-	3.83 to 6.40	<0.001
Ln(Age)	-0.30	-0.81 to -0.22	0.001
Ln(ACR)	-0.31	-0.16 to -0.04	0.001

Dependent variable: Ln(25OHD). Independent variables included in the first step: Ln(Age), Ln(eGFR), Ln(iPTH), Ln(PO₄), Ln(AP), Ln(ACR), Ln(CRP). Adjusted R² = 0.144, p<0.001. N=115. 95% CI: 95% confidence interval; 25OHD: serum 25-hydroxy-vitamin D; eGFR: estimated glomerular filtration rate; iPTH: serum intact parathyroid hormone; PO₄: serum phosphate; AP: serum total alkaline phosphatase; ACR: urinary albumin-to-creatinine-ratio; CRP: C-reactive protein

DISCUSSION

During the past two decades, several epidemiological studies on the prevalence of bone and mineral disorders in CKD have been carried out, but only a minority focused on non-dialysis chronic kidney disease patients, mainly non-referred populations under general practitioners care, with the diagnosis of CKD established based solely on the estimated GFR (7, 9). The only published research in Romanian pre-dialysis patients of which we are aware until now was conducted in a clinic in Timisoara, and included thirty patients with kidney failure (eGFR <10mL/min), without a control group (10). Therefore, the main merit of the current study consists in being the first in our country to investigate a group of over one hundred non-dialysis patients with a broad diversity of kidney function decline (eGFR between 7 - 71 mL/min/1.73m²), in comparison to non-CKD matched subjects.

Figure 3. Correlation of serum intact parathyroid hormone with estimated glomerular filtration rate in chronic kidney disease subjects (N=115). GFR: glomerular filtration rate; iPTH: intact parathyroid hormone.

Figure 4. Correlation of serum calcidiol with age and albuminuria in chronic kidney disease subjects (N=115). ACR: urinary albumin-to-creatinine ratio.

Overall, the serum iPTH was two times higher in CKD group than in Controls. Hyperparathyroidism (HPTH) was found in almost two thirds of the CKD patients, and its prevalence sharply increased with the decline in glomerular filtration. The results are similar to previous reports. For example, in the Study for the Evaluation of Early Kidney Disease (SEEK), 90% of the subjects with eGFR <20mL/min/1.73m² had high levels of iPTH and the prevalence of HPTH in early stages of CKD (i.e. at eGFR >80mL/min/1.73m² and between 60-70mL/min/1.73m²) was around 12% and 21%, respectively (11). The almost triple percentage of patients with HPTH in stage 2 CKD of our study can be an overestimation due to the small number of subjects with this level of GFR (only 9 patients).

It must be emphasized that three subjects from the control group also had iPTH values over the upper limit of the lab (between 75 and 98pg/mL), despite the absence of any endocrinological disease and any suspicion of primary hyperparathyroidism. One possible explanation is the identification in many control patients, including those with high parathyroid hormone, of low levels of serum ionic calcium (<4.3mg/dL) which could account for the subclinical stimulation of PTH synthesis. The high prevalent vitamin D deficiency probably has a causal role. Indeed, hyperparathyroidism (defined as serum iPTH above the upper limit recommended by the manufacturer) was reported in almost 20% of a Romanian cohort of postmenopausal women and its prevalence varied inversely with the calcidiol levels (12).

Hyperphosphatemia (PO₄ >5mg/dL) and hypocalcemia (tCa <8.5mg/dL) were present only among the CKD cohort, with an overall prevalence of 11% and 5%, respectively, and both occurred rather

late in the course of CKD. Thus, 93% of patients with high serum phosphate had a glomerular filtration rate below 30mL/min/1.73m². This is conceivable since hyperphosphatemia is mainly the result of decreased renal clearance of phosphate, often encountered as the kidney excretion function deteriorates. Similarly to our results, in a large group of non-dialysis CKD patients, the prevalence of hyperphosphatemia increased in stages 4 and 5 CKD (8% and 30%, respectively) (13). In accordance with another study that reported a prevalence of decreased serum calcium in almost 20% of patients with eGFR <20mL/min/1.73m² (11), hypocalcemia was also a delayed finding, as it was seen only at eGFR <25mL/min/1.73m² in the current cohort. Moreover, two thirds of the subjects with low serum total calcium had kidney failure (stage 5 CKD). Considering the decisive role of ionized calcium in stimulating PTH synthesis, it would be expected that hypocalcemia precedes the rise in serum iPTH in the course of CKD. The same assertion could apply for increased PO₄. However, these were not found in the present or previous (11, 13) studies, suggesting that other mechanisms contribute to the initial rise in parathyroid hormone. For example, postprandial hypercalciuria with episodic, relative hypocalcemia was proposed (14). Also, the trade-off hypothesis which states that HPTH (along with the increased fibroblast growth factor 23 - not investigated here) is a physiologic adaptive mechanism to maintain normal calcium and phosphate equilibrium (15, 16), could explain the earlier increase of iPTH.

Of note, the multivariate analysis in the current CKD cohort found only the glomerular filtration rate as independent determinant of serum parathyroid hormone. This finding is supported by a cross-sectional study in a large, nationally representative sample of adult Korean population, which showed a negative correlation of serum PTH concentration with eGFR, at least in the presence of vitamin D deficit (17). Contrary reports that deny the influence of moderate kidney function decrease on PTH hypersecretion exist (18), but since this later study enrolled only patients with primary hyperparathyroidism its results might not be extended to CKD population because the pre-existent increased PTH synthesis could mask the effects of kidney insufficiency. Furthermore, a strong association between moderate CKD (eGFR 30-59 mL/min/1.73m²) and higher prevalence of elevated serum iPTH levels (>70 pg/mL) was also described after adjustment for serum calcium, phosphorus, and calcidiol in a large, nationally representative sample of US adults (19).

An alternative explanation of the relationship between iPTH and eGFR could be the accumulation of C-terminal fragments of parathyroid hormone as kidney function declines, which was found to account for an increasing proportion of the iPTH cross-immunoreactivity in CKD, between 30% in stage 4 CKD up to 55% in dialysis patients (20). This issue could have different impact on the intact PTH measurements depending on the commercially available kit used for the laboratory assay, as suggested by the direct comparison of six distinct second generation (two antibodies) PTH assays (21). In that study, the Liaison method (which was the used assay in the current research) was considered the most susceptible to measuring the inactive fragments of parathyroid hormone (21).

As concerns the serum calcidiol, the prevalence of nutritional vitamin D deficit (i.e. low levels of 25OHD defined as serum concentration <30 ng/mL) exceeded 90% in both investigated cohorts, without significant differences across CKD stages. These results are not much different than those of large epidemiological studies that evaluated this deficit in other countries. For example, The National Diet and Nutrition Survey in England reported a 5-20% prevalence of vitamin D deficiency (<10ng/mL) and 90% of values <32ng/mL (22). A somewhat smaller prevalence was recorded between 1988-1994 in the US by the National Health and Nutrition Examination Survey study, which found the deficiency of vitamin D around 1-5% (depending on region, season and age) and a level below 20ng/mL in 10-40% of participants (22). Another cross-sectional cohort study in the US communicated low calcidiol levels (<30ng/mL) in 71% and 83% of CKD stage 3 and 4 patients, respectively, regardless of geographic location (23). Similarly high proportions of vitamin D deficit were reported in non-CKD populations, as low levels of calcidiol (<30ng/mL) were observed in 87% of young healthy women in a sunny country like Saudi Arabia (24), more than 90% of young (35±8 years old) healthy care-givers in Romania (25), and around 83% in Romanian postmenopausal women (12). Another study from Romania found an overall reduced vitamin D in 59% of a large cohort of wide age and geographical distribution range, especially in the elderly (four out of five octogenarians), women and wintertime (26). Multiple reasons for the high prevalence of hypovitaminosis D in our subjects can be supposed, from the reduced sunlight exposure of an elderly population with a significant number of comorbidities, in a country with temperate-continental

climate, to the presumable shortage of vitamin D from the restricted diet.

The predictors of reduced serum calcidiol in the presently studied CKD patients were of older age and had a higher albuminuria (as measured by the urinary albumin-to-creatinine ratio). The high risk of hypovitaminosis D in the elderly is widely accepted, mainly due to both the reduced endogenous production because of restricted outdoor activities which results in low sunlight exposure concomitant with the impaired capacity of the skin to generate vitamin D, and the reduced exogenous dietary intake (27, 28). More interesting is the inverse relationship with albuminuria, which could be a reciprocal linkage. Firstly, it was hypothesized that the urinary loss of vitamin D-binding protein (VDBP) and calcidiol bound to it in proteinuric kidney diseases (especially with nephrotic syndrome) could play a role in the low serum 25OHD levels (29). However, more recently this mechanism was questioned since it was shown that the antiproteinuric treatment did not influence the serum calcidiol despite the reduction of urinary VDBP urinary concentrations (30). Moreover, nephrotic syndrome was an exclusion criterion in the current study. On the other hand, since podocytes (the main native kidney cells involved in the pathogenesis of proteinuria) express both 1- α -hydroxylase and vitamin D receptors, a potential involvement of vitamin D in maintaining their normal function and, consequently, preventing proteinuria is plausible (31). Indeed, a stepwise increase in the prevalence of albuminuria with decreasing quartiles of serum calcidiol, even after multivariate adjustment for confounding factors, was reported (32). Therefore, the observed association in our study could rather express the influence of vitamin D deficiency on proteinuria than vice versa.

The current study has some limitations. Thus, its cross-sectional design makes impossible to establish causal relationships. Secondly, the characteristics of the enrolled subjects (relatively aged, selected from patients who attended nephrology tertiary care settings in the southern regions of Romania) preclude the generalizability of the results to other type of populations. Also, the low number of subjects with stage 2 CKD could weaken the reliability of the findings for the chronic kidney disease patients with mild decline in glomerular filtration. Finally, some biomarkers of mineral and bone metabolism (like 1,25-dihydroxy-vitamin D, osteocalcin, osteoprotegerin, and bone-derived markers of collagen synthesis and breakdown) were

not measured. However, the prospective recruitment with carefully defined CKD (based on repeated eGFR and albuminuria measurements), the enrollment of a control group, and the fact that all biochemical measurements were performed in the same laboratory are strengths of the study. Concerning the lack of data about serum calcitriol levels, this would have minimal impact on the results, since it is in line with KDIGO recommendations which stated that calcidiol is the best index of vitamin D status, while the measurement of 1,25-dihydroxy-vitamin D is less relevant because its half-life is short, its levels are much lower and are influenced by various factors, and the assays are not well standardized (2). On the other hand, newer biomarkers of bone turnover are not sufficiently validated in CKD populations yet (2).

In conclusion, the prevalence of nutritional deficit of vitamin D seems to be very high in older adults from southern Romania, regardless of the presence and severity of chronic kidney disease, but in relation with age and the degree of albuminuria. Abnormalities of all the other investigated biochemical parameters of mineral metabolism (calcemia, phosphatemia, serum iPTH) worsen as glomerular filtration declines in non-dialysis CKD patients who did not previously receive calcium salts and vitamin D derivatives. Secondary hyperparathyroidism was recorded early in the course of CKD, before the appearance of hyperphosphatemia and hypocalcemia, which manifested only in advanced stages of non-dialysis CKD. Therefore, monitoring serum calcium and phosphate is insufficient for early detection of the CKD-associated mineral and bone disorder, and serum parathyroid hormone should be measured in all patients with chronic kidney disease as early as stage 2, at their first presentation in the nephrology department.

Conflict of interest

The authors declare that they have no conflict of interest concerning this article.

References

1. Martin JK, Gonzalez AE. Metabolic bone disease in chronic kidney disease. *J Am Soc Nephrol*. 2007; 18(3):875-885.
2. KDIGO clinical practice guidelines for the diagnosis, evaluation, prevention, and treatment of chronic kidney disease-mineral and bone disorder (CKD-MBD). *Kidney Int*. 2009; 76(suppl 113):S1-S130.
3. Moe SM, Drücke T, Lameire N, Eknoyan G. Chronic kidney disease – mineral-bone disorder: A new paradigm. *Adv Chronic Kidney Dis*. 2007; 14(1):3-12.

4. Floege J, Kim J, Ireland E, Chazot C, Druke T, de Francisco A, Kronenberg F, Marcelli D, Passlick-Deetjen J, Scherthaner G, Fouqueray B, Wheeler DC, pentru ARO Investigators. Serum iPTH, calcium and phosphate, and the risk of mortality in a European haemodialysis population. *Nephrol Dial Transplant*. 2011; 26(6):1948-1955.
5. Chartsrisak K, Vipattawat K, Assanatham M, Nongnuch A, Ingsathit A, Domrongkitchaiporn S, Sumethkul V, Distha-Banchong S. Mineral metabolism and outcomes in chronic kidney disease stage 2-4 patients. *BMC Nephrol*. 2013; 14:14.
6. Chirali S, Mattin C, Belozeroff V, Perrault L, Mitchell D, Gioni I. Impact of mineral and bone disorder on healthcare resource use and associated costs in the European Fresenius medical care dialysis population: a retrospective cohort study. *BMC Nephrol*. 2012; 13:140.
7. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. *Kidney Int*. 2013; Suppl 3(1):S1-S150.
8. Ravine D, Gibson RN, Walker RG, Sheffield LJ, Kincaid-Smith P, Danks DM. Evaluation of ultrasonographic diagnostic criteria for autosomal dominant polycystic kidney disease 1. *Lancet*. 1994; 343(8901):824-827.
9. Scherpier-de Haan ND, Vervoort GM, Van Weel C, Mulder J, Wetzels JF, de Grauw WJ. Abnormal serum parathyroid hormone, calcium or phosphate in patients with chronic kidney disease in primary care. *Austin J Nephrol Hypertens*. 2014;1(6): 1027.
10. Mihăilescu D, Velciov V. Specificities of the phospho-calcic metabolism in patients with chronic renal disease in initiating the assisted substitution of the renal function in a hemodialysis center in western Romania. *J Exp Med Surgical Res*. 2010; XVII(2):112-120.
11. Levin A, Bakris GL, Molitch M, Smulders M, Tian J, Williams LA, Andress DL. Prevalence of abnormal serum vitamin D, PTH, calcium, and phosphorus in patients with chronic kidney disease: Results of the Study to Evaluate Early Kidney Disease. *Kidney Int*. 2007; 71(1):31-38.
12. Grigorie D, Sucaliuc A, Ivan M, Neacsu E, Popa O, Diaconescu A. High prevalence of vitamin D deficiency in 1048 Romanian women with postmenopausal osteoporosis. *Acta Endo (Buc)*. 2008;4(1):33-45.
13. Moranne O, Froissart M, Rossert J, Gauci C, Boffa JJ, Haymann JP, M'rad MB, Jacquot C, Houillier P, Stengel B, Fouqueray B for NephroTest Study Group. Timing of onset of CKD-related metabolic complication. *J Am Soc Nephrol*. 2009; 20(1):164-171.
14. Isakova T, Gutierrez O, Shah A, Castaldo L, Holmes J, Lee H, Wolf M. Postprandial mineral metabolism and secondary hyperparathyroidism in early CKD. *J Am Soc Nephrol*. 2008; 19(3):615-623.
15. Bricker NS. On the pathogenesis of the uremic state. An exposition of the "trade-off" hypothesis. *N Engl J Med*. 1972; 286(20):1093-1099.
16. Gutiérrez OM. Fibroblast growth factor 23 and disordered vitamin D metabolism in chronic kidney disease: Updating the "trade-off" hypothesis. *Clin J Am Soc Nephrol*. 2010; 5(9):1710-1716.
17. Han SW, Kim SJ, Lee DJ, Kim KM, Joo NS. The relationship between serum 25-hydroxyvitamin D, parathyroid hormone and the glomerular filtration rate in Korean adults: The Korea National Health and Nutrition Examination Survey between 2009 and 2011. *Korean J Fam Med*. 2014; 35(2):98-106.
18. Tassone F, Gianotti L, Emmolo I, Ghio M, Borretta G. Glomerular filtration rate and parathyroid hormone secretion in primary hyperparathyroidism. *J Clin Endocrinol Metab*. 2009; 94(11):4458-4461.
19. Muntner P, Jones TM, Hyre AD, Melamed ML, Alper A, Raggi P, Leonard MB. Association of serum intact parathyroid hormone with lower estimated glomerular filtration rate. *Clin J Am Soc Nephrol*. 2009; 4(1):186-194.
20. Brossard JH, Lepage R, Cardinal H, Roy L, Rousseau L, Dorais C, D'Amour P. Influence of glomerular filtration rate on non-(1-84) parathyroid hormone (PTH) detected by intact PTH assays. *Clin Chem*. 2000; 46(5):697-703.
21. La'ulu SL, Roberts WL. Performance characteristics of six intact parathyroid hormone assays. *Am J Clin Pathol*. 2010; 134(6):930-938.
22. Prentice A. Vitamin D deficiency: A global perspective. *Nutr Rev*. 2008; 66(suppl 2):S153-S164.
23. LaClair RE, Hellman RN, Karp SL, Kraus M, Ofner S, Li Q, Graves KL, Moe SM. Prevalence of calcidiol deficiency in CKD: A cross-sectional study across latitudes in the United States. *Am J Kidney Dis*. 2005; 45(6):1026-1033.
24. Hasanato R, Al-Mahboob A, Al-Mutairi A, Al-Faraydi J, Al-Amari K, AL-Jurayyad R, Mohamed S. High prevalence of vitamin D deficiency in healthy female medical students in central Saudi Arabia: Impact of nutritional and environmental factors. *Acta Endo (Buc)*. 2015;11(2):257-261.
25. Porojnicu AC, Moroti-Constantinescu R, Laslau A, Lagunova Z, Dahlback A, Hristea A, Moan J. Vitamin D status in healthy Romanian caregivers and risk of respiratory infections. *Public Health Nutr*. 2012;15(11):2157-2162.
26. Chirita-Emandi A, Socolov D, Haivas C, Calapis A, Gheorghiu C, Puiu M. Vitamin D Status: A Different Story in the Very Young versus the Very Old Romanian Patients. *PLoS One*. 2015;10(5):e0128010.
27. Hagenau T, Vest R, Gissel TN, Poulsen CS, Erlandsen M, Mosekilde L, Vestergaard P. Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: An ecologic meta-regression analysis. *Osteoporos Int*. 2009; 20(1):133-140.
28. Lips P. Vitamin D deficiency and secondary hyperparathyroidism in the elderly: Consequences for bone loss and fractures and therapeutic implications. *Endocrine Rev*. 2001; 22(4):477-501.
29. Bhan I, Thadhani R. Calcium, phosphate, and vitamin D in kidney disease. In Mitch WE, Ikizler TA, eds. *Handbook of nutrition and the kidney*. 6th ed. Philadelphia, Lippincott, Williams & Wilkins, 2010:50-57.
30. Doorenbos CR, de Cuba MM, Vogt L, Kema IP, van den Born J, Gans RO, Navis G, de Borst MH. Antiproteinuric treatment reduces urinary loss of vitamin D-binding protein but does not affect vitamin D status in patients with chronic kidney disease. *J Steroid Biochem Mol Biol*. 2012; 128(1-2):56-61.
31. Pérez-Gómez MV, Ortiz-Ardúan A, Lorenzo-Sellares V. Vitamin D and proteinuria: A critical review of molecular bases and clinical experience. *Nefrologia*. 2013; 33(5):716-726.
32. Agarwal R. Vitamin D, proteinuria, diabetic nephropathy, and progression of CKD. *Clin J Am Soc Nephrol*. 2009; 4(9):1523-1528.