Important abnormalities of bone mineral metabolism are present in patients with coronary artery disease with mild decrease of estimated glomerular filtration rate

Short title: CKD-MBD in coronary artery disease patients

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ABSTRACT

Chronic kidney disease mineral bone disorder (CKD-MBD) is characterized by increased circulating levels of parathormone (PTH) and fibroblast growth factor-23 (FGF23), bone disease and vascular calcification, and is associated with adverse outcomes. We studied the prevalence of mineral metabolism disorders, and the potential relationship between decreased estimated glomerular filtration rate (eGFR) and CKD-MBD in coronary artery disease patients in a cross-sectional study of 704 outpatients 7.5±3.0 months after an acute coronary syndrome. Mean eGFR (CKD-EPI formula) was 75.8±19.1 ml/min/1.73 m^2. Our patients showed lower calcidiol plasma levels than a healthy cohort from the same geographical area. In the case of men, this finding was present in spite of similar creatinine levels in both groups and older age of the healthy subjects. Most patients (75.5%) had an eGFR<90 ml/min/1.73 m^2 (G2-G5 CKD stages) with 50% of cases showing values of 60-89 ml/min/1.73 m^2 (G2). PTH (r=-0.3329, p<0.0001) and FGF23 (r=-0.3641, p<0.0001) inversely correlated with eGFR, while 25OH vitamin D levels and serum phosphate levels did not. Overall, PTH levels were above normal in 35% of patients. This percentage increased from 19.4% in G1, to 33.4% in G2 and 56.6% in G3-5 (p<0.001). At multivariate analysis, eGFR and 25OH vitamin D levels were the main independent determinants of serum PTH. Mean FGF23 levels were 69.9 (54.5-96.3) RU/ml, and 33% of patients had FGF23>85.5 RU/mL. This value was 18.4% in G1, 30.0% in G2, and 59.2% in G3-5 (p<0.001). In multivariate analysis, eGFR was the main predictor of PTH and FGF23 levels. Increased phosphate levels were present in 0.7% of the whole sample, 0% in G1, 0.3% in G2 and 2.8% in G3-5 (p=0.011). Ninety-nine percent of cases showed calcidiol insufficiency without significant differences among the different degrees of eGFR. In conclusion, in patients

with coronary artery disease there is a large prevalence of increased FGF23 and PTH levels. These findings have an independent relationship with decreased eGFR, and are evident at eGFR 60-89 ml/min/1.73 m^2. Then, mild decrements in eGFR must be taken in consideration by the clinician, since they are associated with progressive abnormalities of mineral metabolism.

KEY WORDS: Glomerular filtration rate, FGF23, PTH, vitamin D, coronary artery disease

INTRODUCTION

The KDIGO 2012 Clinical Practice Guidelines for the Evaluation and Management of Chronic Kidney Disease define chronic kidney disease (CKD) as the presence for more than 3 months of abnormalities of kidney structure or function, with implications for health [1]. CKD is classified based on cause, glomerular filtration rate (GFR) category, and albuminuria category (CGA classification). Six GFR categories are recognized: G1 (Normal or high, ≥90 ml/min/1.73 m^2), G2 (Mildly decreased, 60-89) G3a (Mildly to moderately decreased, 45-59), G3b (Moderately to severely decreased, 30-44), G4 (Severely decreased 15-29) and G5 **(**Kidney failure <15). CKD is defined as either an eGFR <60 ml/min/1.73 m^2 or evidence of kidney injury such as a urinary albumin/creatinine ratio (UACR) >30 mg/g [1]. The basis of this classification is the increased relative risk of adverse outcomes associated with either a eGFR <60 ml/min/1.73 m^2 or a urinary albumin/creatinine ratio >30 mg/g [1]. These adverse outcomes include all-cause and cardiovascular mortality, progressive CKD and acute kidney injury. Interestingly, while renal outcomes are clearly associated with these two cut-off points (eGFR <60 ml/min/1.73 m^2 or UACR >30 mg/g) an increased risk of all-cause and cardiovascular death is already observed in patients with reduced eGFR (eGFR category G2, 60-89 ml/min/1.73 m^2) even when UACR is below <30 mg/g [1]. KDIGO 2012 recommends reporting eGFR in adults using the 2009 CKD Epidemiology Collaboration (CKD-EPI) creatinine equation [1], since it provides an accurate estimate of eGFR in all GFR categories [1, 2]. In patients with heart failure, the CKD-EPI equations were superior to the older Modification of Diet in Renal Disease (MDRD) equation for predicting mortality, especially in patients with GFR >60 mL/min [3]. In this regard, the MDRD equation was generated from CKD patients participating in a clinical trial and is not accurate for patients with GFR >60 ml/min. In the past decade many countries followed the recommendation that, whenever serum creatinine is

measured, MDRD-derived eGFR is reported simply as >60 ml/min/1.73 m^2 when it is above this value, providing only the specific result when it is <60 ml/min/1.73 m^2 [4]. Together with the lack of routine UACR assessment, reporting eGFR as >60 ml/min/1.73 m^2 based on the MDRD equation may lead to under diagnosis of CKD by many non-nephrologists. This may deprive patients from early diagnosis and management of CKD complications, such as mineral and bone disorders (CKD-MBD). CKD-MBD is an early and progressive complication of CKD associated to accelerated vascular calcification, left ventricular hypertrophy and increased cardiovascular morbidity and mortality [5, 6]. The earliest manifestation of CKD-MBD is associated with disruption of phosphate homeostasis, vascular calcification and accelerated aging [7-9].

In this paper, the novelty point is that we have studied the status of the components of mineral metabolism in patients with stable coronary artery disease from the BACS & BAMI (Biomarkers in Acute Coronary Syndrome & Biomarkers in Acute Myocardial Infarction) studies [10], by assessing eGFR by the CKD-EPI equation, according to the most recent CKD guidelines [1]. We have divided the population in three groups according to GFR (<60, 60-89, and ≥90 ml/min/1.73 m^2) showing that mild decrements in GFR have an impact on CKD-MBD parameters. This suggests that all patients with coronary artery disease should be screened for CKD using the CKD-EPI equation, to detect those with an eGFR of 60-89 ml/min/1.73 m^2.

MATERIALS AND METHODS

Study Design

The research protocol was approved by the ethics committees of the hospitals and all patients signed informed consent documents. The BACS & BAMI studies enrolled patients admitted to four hospitals in the area of Madrid (Spain) with either non-ST elevation acute coronary syndrome or ST-elevation acute myocardial infarction [1]. Non-ST elevation acute coronary syndrome was defined as rest anginal pain lasting more than 20 minutes in the previous 24 hours, or new-onset class III-IV angina, along with transient ST depression or T wave inversion in the electrocardiogram considered diagnostic by the attending cardiologist and/or troponin elevation. ST-elevation myocardial infarction was defined as symptoms compatible with angina lasting more than 20 minutes and ST elevation in two adjacent leads in the electrocardiogram without response to nitroglycerin, and troponin elevation. Exclusion criteria were age over 85 years, coexistence of other significant cardiac disorders except left ventricular hypertrophy secondary to hypertension, coexistence of any illness or toxic habits that could limit patient survival, impossibility to perform revascularization when indicated, and subjects in whom follow-up was not possible. In order to avoid variability of findings due to an excessive heterogeneity in the intervals between the acute event and blood sampling, patients not clinically stable at day six of the index event were excluded.

Between July 2006 and April 2010, 1,898 patients were discharged from the study hospitals with a diagnosis of non-ST elevation acute coronary syndrome or ST-elevation acute myocardial infarction. Eight hundred thirty-eight patients were enrolled in the study. The remaining patients were not enrolled due to age over 85 years (17.3%), presence of disorders or toxic habits limiting survival (29.0%), impossibility to perform cardiac revascularization (14.5%), coexistence of other significant cardiopathy (6.8%),

impossibility to perform follow-up (12.0%), clinical instability beyond day 6 of the index event (9.1%), refusal to participate in the study (2.0%), and impossibility of the investigators to enroll them (9.3%). Of 838 patients enrolled, 7 died after discharge and 709 returned for an outpatient visit and blood sampling between January 2007 and February 2011 (between six and twelve months after discharge). Five patients were lost at follow-up leaving a total of 704 patients for analysis.

Biochemical studies

At the outpatient visit, a complete set of clinical variables were recorded and 12-hour fasting venous blood samples were collected in EDTA, centrifuged at 2,500 g for 10 minutes and plasma stored at -80°C in the biobank of IIS-Fundación Jiménez Díaz until assessment of 25 OH vitamin D (25OHD), fibroblast growth factor-23 (FGF23) and intact parathormone (PTH) at the laboratory of Nephrology and Mineral Metabolism at the Gómez-Ulla hospital. The remaining analytes were assessed at the Vascular Pathology and Biochemistry Laboratories at Fundación Jiménez Díaz. The investigators who performed the laboratory studies were blinded to clinical data. Plasma 25OHD levels were quantified by chemiluminescent immunoassay (CLIA) on the LIAISON® XL analyzer (LIAISON 25OH Vitamin D total Assay DiaSorin, Saluggia, Italy), FGF23 was measured by an enzyme-linked immunosorbent assay which recognizes epitopes within the carboxyl-terminal portion of FGF23 (Human FGF23, C-Term, Immutopics Inc, San Clemente, CA), intact PTH was analyzed by a second-generation automated chemiluminescent method (Elecsys 2010 platform, Roche Diagnostics, Mannheim, Germany), phosphate was determined by an enzymatic method (Integra 400 analyzer, Roche Diagnostics, Mannheim, Germany). High-sensitivity C-reactive protein (hs-CRP) was assessed by latex-enhanced immunoturbidimetry (ADVIA 2400 Chemistry System, Siemens, Germany). Kidney function was estimated

by the CKD-EPI eGFR equation [11].

Statistical Analysis

Quantitative data that followed a normal distribution (Kolmogorov-Smirnov test) are presented as mean±standard deviation, and those not normally distributed are displayed as median (interquartile range). Qualitative variables are displayed as percentages. 25OHD levels were assessed as four clinically relevant categories: ≤10.0 ng/ml (severe deficiency), 10.01-20.00 ng/ml (moderate deficiency), 20.01-30.0 ng/ml (insufficiency/suboptimal levels), and >30 ng/ml (sufficiency). To assess correlations, some values were log-transformed and Pearson´s "r" was performed. Multivariate linear regression models were used to identify factors independently associated with serum PTH or FGF23. Models were built using forward stepwise procedures in order to maximize r-squared with the smallest number of predictor variables. Clearly asymmetric variables were log transformed. Results are expressed as coeficients and 95% confidence interval, "p" values and adjusted "r" squared value. The statistical significance of variables in the models was assessed by ANOVA for variables with normal distribution and Kruskal-Wallis tests for variables not normally distributed, respectively. Analyses were performed with SPSS 19.0 (SPSS Inc., New York) and Instat 3.10 (Graphpad Software Inc., La Jolla, CA).

RESULTS

Renal function and CKD-MBD parameters were assessed in 704 outpatients 7.5±3.0 months after a non-ST-elevation acute coronary syndrome or a ST-elevation acute myocardial infarction. **Table 1** shows epidemiological and analytical parameters for the whole cohort and for 3 different KDIGO 2012 eGFR categories (G1, G2, and G3-5). Mean eGFR estimated by the CKD-EPI formula was 75.8±19.1 ml/min/1.73 m^2. A majority of patients (75.5 %) had a low eGFR (GFR categories G2-G5, <90 ml/min/1.73 m^2). Indeed, over 50% of patients had an eGFR 60-89 ml/min/1.73 m2 (G2). In the absence of albuminuria data, G2 patients are not considered as having CKD [1]. Compared to patients with normal eGFR (G1), patients with decreased eGFR were older, less frequently male and had higher hsPCR values suggesting systemic inflammation (**Table 1**). Patients with decreased eGFR also had more prevalence of cerebrovascular events, peripheral artery disease, atrial fibrillation and reduced ejection fraction.

Only 4 patients (0.6%) were taking vitamin D supplements and none was taking cinacalcet or phosphate binders. Thus, the cohort can be considered to reflect natural history CKD-MBD parameters in coronary artery disease patients. Overall, 25OHD, PTH, FGF23 and serum phosphate levels differed between the three eGFR categories (G1, G2, and G3-5, **Table 1**).

However, only PTH and FGF23 progressively changed according to progressively lower eGFR (**Figure 1**). This is in accordance to our current understanding of CKD-MBD. Thus, while there are CKD-associated factors (e.g. proteinuria with loss of protein-bound vitamin D) that may impact on 25OHD levels, the main risk factors for low 25OHD levels (low sun exposure and low vitamin D intake) are not CKD-specific [12]. In this regard, mean 25OHD levels were 19.61 ± 8.23 ng/ml, 90% of patients in all eGFR categories had 25OHD insufficiency or

deficiency (<30 ng/ml, **Table 1**), and there was no correlation between 25OHD levels and eGFR (**Table 2**). Serum phosphate levels did not correlate with eGFR either (**Table 2**). In this regard, the vast majority of patients had normal serum phosphate levels and only 5 (0.7%) had serum phosphate >4.5 mg/dl. This is not surprising since, given the toxicity of excess phosphate [13, 14], compensatory mechanisms such as increased serum FGF23 and PTH levels are activated very early in the course of CKD and hyperphosphatemia is typically not observed until eGFR categories G4 and mainly G5 [2, 15]. Thus, the main CKD-MBD associated changes in the present coronary artery disease cohort were observed for PTH and FGF23.

Median serum PTH levels were 59.7 (45.5-77.5) pg/ml and median PTH progressively increased as eGFR decreased as assessed by eGFR category (**Table 1, figure 1**) or as a continuous variable (r -0.3329, p<0.0001, **table 2, figure 2**). Overall, 35% of study patients displayed above normal PTH levels (>74 pg/ml according to normal range for the lab). Among patients with normal renal function (eGFR category G1), 19% had high PTH levels. In eGFR category G1, low 25OHD levels are inversely correlated with drivers of high PTH levels (**Table 2**, r -0.300, p <0.0001), although eGFR was also a contributor (r -0.164, p 0.0326). We should remember that the original definition of normal 25OHD levels was based on their association with normal PTH levels [16]. In this regard, 25OHD levels also inversely correlate with serum PTH levels in all individual eGFR categories (**Table 2**). Furthermore, in multivariate analysis, eGFR and 25OHD levels were the main independent determinants of serum PTH. Serum phosphate (negative correlation), hsCRP (negative correlation) and FGF23 (positive correlation) were also independently correlated with PTH (**Table 3a**).

Median serum FGF23 levels were 69.9 (54.5-96.3) RU/ml, and 33% of the patients had FGF23 >85.5 RU/mL, considered to confer a higher risk of CKD progression [17]. Already in eGFR category G1, 18% of patients have FGF23 values

above this limit. The driver of these early changes was unclear and could be related to variables not assessed in the present study, such as dietary phosphate and low kidney Klotho levels [8, 9, 18-20]. Both within eGFR categories G2 and G3-G5 and for the overall cohort, a clear inverse correlation of FGF23 levels with eGFR was observed. Indeed, in multivariate analysis (**Table 3b**), eGFR was the main predictor of FGF23 levels. Additional independent predictors were gender, phosphate, hsCRP and PTH. Contrary to the findings for PTH, phosphate and hsCRP were directly correlated to FGF23 levels. **Table 4** shows the distribution of eGFR and mineral metabolism parameters across the different ranges of age.

The PTH/phosphate and FGF23/phosphate ratios increased as eGFR category worsened (**Table 1**), reflecting the need of higher concentrations of phosphaturic hormones to maintain normal phosphate levels as glomerular phosphate filtration decreases and progressive resistance to their kidney effects develops[19].

Finally we compared our data on calcidiol plasma levels with those of a healthy cohort with 657 men and 1,154 women recently published in Spain by Olmos et al [21]. In men, as expected, calcidiol values were lower in our study than in the healthy cohort (20.2±8.4 vs 23.5±7.7 ng/ml; p<0.001). This was so in the absence of significant differences in creatinine plasma levels (1.09±0.33 vs 1.08±0.2 mg/dl; p=0.503). Even more, our patients were younger than those of the healthy cohort (59.8±11.7 vs 64.6±8.4 years; p<0.001). With regard to women, our patients also had lower calcidiol levels (17.9±7.3 vs 22.1±7.9 mg/dl; p<0.001). However, opposite to what we observed in men, age (66.4±12.7 vs 63.2±9.8 years; p<0.001) and plasma creatinine levels were higher than those of healthy subjects (0.94±0.36 vs 0.90±0.20 mg/dl; p=0.002)

DISCUSSION

The main findings of this study are that most patients with coronary artery disease have a decrease in calcidiol plasma levels, which is more prevalent than in healthy subjects of the same geographical area. Of special interest, in the subgroup of men this finding was present despite both similar creatinine plasma levels in both populations. In addition, the age of our patients was significantly higher than that of the healthy cohort, meaning that the lower calcidiol levels found in men with coronary disease may not be attributed to a poorer renal function or to an older age. It must be emphasized than patients with life threatening comorbidities, among which were those on hemodialysis, were excluded from our study.

Of great interest, the abnormalities in mineral metabolism were present at CKD-MBD with eGFR<90 ml/min/1.73 m^2. There is a high prevalence of reduced eGFR in patients with coronary artery disease. While the current data do not allow a firm diagnosis of CKD for patients on eGFR categories G1 and G2, due to the lack of confirmation in a second dataset three months apart and the lack of availability of UACR values (as is frequently the case for cardiology patients), we provide evidence that these low values of eGFR, even within eGFR category G1, may be adversely affecting CKD-MBD parameters such as PTH and FGF23 and, thus, may be potentially contributing to findings frequently observed in coronary artery disease patients such as left ventricular hypertrophy, vascular and valve calcification and high mortality rates [20,22-32].

CKD is present in a substantial proportion of patients with coronary artery disease. In pooled data from five international multicenter trials 16% of 13,307 patients with acute coronary syndrome had eGFR <60 mL/min/1.73 m^2 [33]. More recently, the CLARIFY registry showed that 22.1% of patients with stable coronary artery disease have an eGFR below this level [34], a similar proportion to the 20.3% described in the present study. In addition, 76% of patients had eGFR assessed by CKD-EPI under 90

ml/min/1.73 m^2. This cut-off point includes a much higher percentage of patients than the usual limit of 60 ml/min/1.73 m^2 to define CKD on the basis of eGFR alone (60 ml/min/1.73 m^2). However, contrary to the common perception that mild decreases in eGFR (G1, G2) may be associated with treatable biochemical abnormalities, we describe the frequent occurrence of increased serum PTH and FGF23 levels even at these early stages. Indeed, PTH and FGF23 negatively correlated with eGFR in the whole cohort, where eGFR was the main determinant of PTH and FGF23 levels in multivariate analysis. Furthermore, eGFR correlated with PTH levels even in eGFR category G1 and with FGF23 levels from eGFR category G2, suggesting an impact of eGFR on CKD-MBD even at eGFR levels that are often considered "normal" for age.

Patients with CKD gradually lose the capacity to excrete phosphate due to reduced nephron number, which results in a trend towards a positive phosphate balance [19]. Mild decrements in eGFR (eGFR category G2) are already associated with decreased glomerular filtration of phosphate and compensatory responses to maintain phosphate homeostasis, such an increased FGF23 and PTH levels [8, 19]. In this regard, high FGF23 levels indicate a physiological response to a state of phosphate excess that is due to an increase in dietary intake or a reduction in kidney excretion and may be associated with soft tissue phosphate deposition, even in the absence of high serum phosphate levels. Uremia favors vascular deposition of phosphate [35]. FGF23 protects from excess phosphate by decreasing kidney tubular phosphate reabsorption, thus promoting urinary phosphate excretion, and by inhibiting activation of 25OHD to 1,25 $(OH)_2$ vitamin D in kidney tubular cells [36,37]. The resulting low 1,25 $(OH)_2$ vitamin D levels impair calcium and phosphate absorption in the gut, thus protecting from dietary phosphate excess. However, low 1,25 $(OH)_2$ vitamin D levels promote a trend towards hypocalcemia and increased PTH concentration [19,38]. The high prevalence of nutritional and sun-induced vitamin D deficiency in the general population and in

coronary artery disease patients could cause a decrease in 25OHD availability for activation in the kidneys [39]. As a result of high FGF23 and PTH levels, serum phosphate levels remain within normal limits up to very advanced CKD stages [5, 15].

Patients with reduced GFR or CKD are at increased risk of adverse cardiac outcomes [15,40,41]. In patients with no CKD, bone mineral disorders have also been linked to the severity of coronary artery disease, as seen on coronary angiography [42], as well as to increased carotid intima-media thickness [43] and ventricular hypertrophy [44]. Serum levels of 25OHD, PTH, phosphate and FGF23 have been associated with prevalent and incident cardiovascular disease, suggesting a role for bioregulators of bone and mineral metabolism in cardiovascular health. Observational studies have clearly linked high FGF23 and low 25OHD levels with adverse patient outcomes [12,20,22-28,45-47]. Studies linking high serum PTH with adverse outcomes are more controversial [29-32]. Since in the present study 25OHD levels were not related to eGFR and high phosphate levels were very uncommon, we will only discuss FGF23 and PTH.

Higher FGF23 levels are independently associated with mortality and cardiovascular events in coronary patients and in the community as well as with risk of stroke independently of renal function [17,20,22-28]. In the Heart and Soul Study, patients in the highest tertile of FGF23 had an 83% increase in cardiovascular events [22]. The increased risk conferred by higher FGF23 levels may be related to its status as a marker of phosphate excess, as a marker of Klotho deficiency, or to direct adverse effects of FGF23 promoting left ventricular hypertrophy or vascular calcification [6, 9, 19, 20, 26]. In his regard, although serum phosphate levels were mostly within normal limits in our study, there is a graded independent relation between higher levels of serum phosphate and the risk of death and cardiovascular events in people with prior

myocardial infarction, most of whom had serum phosphate levels within the normal range [48] as well as in other non-CKD populations [13,14,49,50].

In our study, the multivariate analysis showed that eGFR is the main determinant of serum FGF23 levels. In addition, phosphate, hsCRP and PTH were directly correlated to FGF23 levels. These associations may be causal, according to knowledge gathered from experimental animal models. Thus, excess phosphate is the main physiological stimulus increasing FGF23 levels [19,50]. In addition, systemic inflammation lowers kidney Klotho [51] and decreased kidney Klotho alone may result in higher FGF23 levels [9]. The existence of these independent drivers of FG23 levels may explain the presence of high FGF23 levels in 18% of patients with eGFR≥90 ml/min/1.73 m^2. Thus either systemic inflammation or excess dietary phosphate may result in increased FGF23 secretion. In this regard, the multivariate model only explained 18% of the FGF23 variability. These data suggest an important role of additional factors, potentially including an excess of phosphate ingestion.

Elevated PTH has been associated with a greater prevalence and incidence of cardiovascular risk factors and predicts a greater likelihood of prevalent and incident disease and mortality [28], even in patients with stable coronary artery disease [29]. In the Uppsala Longitudinal Study in Adult men (ULSAM) community-based study higher PTH was independently associated with a 38% greater risk of cardiovascular mortality [30]. However, no association between PTH and the risk of coronary heart disease was observed in the Health Professionals Follow-up Study in 51,529 healthy professionals over 40-75 years of age during 10 years follow-up [31] and a recent meta-analysis did not show an association between PTH and all-cause mortality in CKD [32]. In our study there was a high incidence of elevated PTH levels in coronary patients. In multivariate analysis low eGFR and low 25 OH D vitamin D levels were the main independent determinants of higher serum PTH. In addition, there was an independent negative

correlation between serum phosphate and PTH. The negative correlation between serum phosphate and PTH levels clearly differs from the positive correlation between serum phosphate and FGF23 levels. This observation is consistent with excess phosphate being the primary driver of FGF23 secretion, while hypocalcemia and low 25OHD levels are the key drivers of PTH secretion. Thus, when PTH is increased in response to low calcium levels or to low vitamin D levels (that also impair phosphate absorption from the gut) the resulting increased urinary phosphate excretion may lead to lower serum phosphate levels. The independent positive correlation between PTH and FGF23 may be the result of complex interactions between both molecules. Thus, FGF23 directly suppresses PTH secretion [52]. However, the trend towards hypocalcemia as a result of FGF23-mediated suppression of vitamin D activation may increase PTH levels [19,38]. Finally, the presence of diabetes correlated inversely with PTH levels. As hyperglycemia is known to suppress PTH secretion [53], it could be hypothesized that this feature could interfere with the development of atherosclerosis in diabetes. However, low serum PTH levels may have also an adverse effect over the cardiovascular system through different mechanisms. Low PTH decreases bone turn-over, and may lead to adynamic bone disease [54], a well-recognized clinical entity in CKD which is associated not only to increased risk of bone fractures but also to vascular calcifications that may at least partially explain the association of this disorder with increased mortality rates. Moreover, diabetes, as well as ageing, has been associated to this disorder [54]. Then, there is no reason to hypothesize that PTH suppression in diabetes could modify favorably the clinical course of diabetes-related atherosclerotic changes.

This study has some limitations. Urine albumin, Klotho, and calcium plasma levels were not measured, and renal echography was not performed. These data could have added important information to this paper.

In conclusion, patients with coronary artery disease show low plasma levels of calcidiol even when compared with a healthy cohort. Even more, in the subgroup of men these results were present without differences in creatinine plasma levels and with coronary patients being younger than healthy controls. In addition, in our population, this decrease in calcidiol and an increase in FGF23 and PTH levels are already evident below eGRF<90 ml/min/1.73 m^2. The current standard in many countries for eGFR assessment is the MDRD formula and actual results are only reported if <60 ml/min/1.73 m^2. This approach results in unawareness by the treating physicians of low eGFR in coronary artery disease patients. We propose following recent KDIGO 2012 guidelines for CKD and implement reporting of actual eGFR values based on the CKD-EPI formula. Further studies are needed that explore whether reducing phosphorus intake and/or supplementing vitamin D will restore normal PTH and FGF23 levels in coronary artery disease patients with reduced eGFR and whether this intervention improves patient outcomes.

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Table 1. Key patient characteristics according to eGFR category (ml/min/1.73 m^2) assessed by the CKD-EPI formula and following the KDIGO eGFR category definition (1)

	All n=704	**Category G1 eGFR≥90 (n=172)**	**Category G2 eGFR 60-89 (n=389)**	**Category G3 -G5 eGFR<60 (n=143)**	**P**
Age (years)	61.4 ± 12.3	51.4 ± 8.6	61.9 ± 11.1	72.1 ± 9.2	**<0.001**
Male Sex (%)	75.3	83.7	77.9	58.0	**<0.001**
Caucasian (%)	97.0	94.2	97.4	99.3	**0.023**
Present Smoker (%)	6.5	10.5	5.1	5.6	0.055
Diabetes (%)	24.6	23.3	22.9	30.8	0.155
Hypertension (%)	65.1	48.3	65.8	83.2	**<0.001**
Dyslipidemia (%)	59.1	58.1	58.6	61.5	0.796
Body Mass index (Kg/m^2)	28.41 (25.71-30.91)	28.5 ± 4.5	29.0 ± 4.6	28.1 ± 4.2	0.111
Cerebrovascular events (%)	3.4	1.2	2.1	9.8	**<0.001**
Peripheral artery disease (%)	3.8	2.9	2.8	7.7	**0.027**
Atrial Fibrillation (%)	4.8	1.2	4.1	11.2	**<0.001**
Ejection fraction <40% (%)	11.8	9.3	10.0	19.6	**0.005**
LAST CORONARY EVENT					
STEMI/NSTEACS (%)	38.6/61.4	38.4/61.6	38.8/61.2	38.5/61.5	0.994
ANALYTIC PARAMETERS					
eGFR (CKD-EPI) (ml/min/1.73m^2)	75.8±19.1	98.9 ± 6.2	76.1 ± 8.2	47.3 ± 10.6	**<0.001**
Glycemia	100 (91.0 - 115.0)	110.3 ± 41.7	107.7 ± 30.8	111.7 ± 44.3	0.525
HDL cholesterol (mg/dl)	42 (36.0 – 49.0)	42.6 ± 10.4	44.3 ± 10.8	44.1 ± 11.7	0.213
LDL cholesterol (mg/dl)	81.0 (66.0 – 96.0)	85.7 ± 28.5	82.7 ± 23.7	81.3 ± 27.0	0.335
Triglycerides (mg/dl)	111 (82.0 – 153.0)	112.5 (82.8 – 151.3)	106 (80.0 – 149.5)	119 (86.0 – 169.5)	0.138
HS C-reactive protein (mg/dl)	1.96 (0.83 – 4.12)	1.91 (0.88 – 3.67)	1.72 (0.78 – 3.71)	2.91 (1.20 – 6.13)	**<0.001**
Calcidiol (ng/dl)	19.6±8.2	18.7 ± 7.5	20.4 ± 8.6	18.6 ± 8.0	**0.023**
Calcidiol range (%)					0.055
≤10 ng/ml	10.9	10.6	10.1	13.3	
10.01 – 20.0 ng/ml	46.4	51.2	44.0	46.9	
20.01 – 30.0 ng/ml	32.3	33.5	32.1	31.5	
>30.0 ng/ml	10.4	4.7	13.7	8.4	
PTH (pg/dl)	59.7 (45.5-77.3)	54.2 (44.1 – 65.9)	58.0 (43.9 – 75.9)	75.2 (53.5 – 99.4)	**<0.001**
PTH > 74 pg/ml (%)	34.9	19.4	33.7	56.6	**<0.001**
FGF23 (RU/dl)	69.9 (54.6-96.2)	65.7 (48.4 – 78.9)	68.1 (53.9 – 89.1)	98.5 (72.5 – 159.0)	**<0.001**
FGF23 >85.5 RU/ml (%)	33.2	18.4	30.0	59.2	**<0.001**
Phosphate (mg/dl)	3.21±0.54	3.25 ± 0.54	3.15 ± 0.51	3.30 ± 0.63	**0.018**
Phosphate > 4.5 mg/ml (%)	0.7	0	0.3	2.8	**0.011**
PTH/phosphate ratio	18.6 (13.8 – 25.2)	16.6 (12.5-21.9)	18.6 (13.7-24.6)	22.8 (16.0-32.4)	**<0.001**
FGF 23/phosphate ratio	22.5 (17.5 – 31.8)	19.6 (15.6-26.0)	22.1 (17.4-29.5)	32.7 (22.5-48.1)	**<0.001**
Calcidiol/phosphate ratio	5.88 (4.31 -7.82)	5.74 (4.3-7.2)	6.03 (4.57-8.16)	5.68 (3.53-7.65)	**0.011**

Categorical variables are presented as percentages, quantitative variables with normal distribution as mean±SD and those not normally distributed as median (interquartile range).

STEMI: ST-Elevation Myocardial Infarction. NSTEACS: Non-ST Elevation Acute Coronary Syndrome; eGFR: estimated Glomerular Filtration Rate; HDL: High-density lipoprotein; LDL: Low-density lipoprotein; HS: High-Sensitivity; PTH: parathormone; FGF-23: Fibroblast Growth Factor-23.

P value refers to the comparison among G1, G2 and G3-5 groups.

Table 2. Univariate correlations between individual CKD-MBD parameters according to eGFR category assessed by the CKD-EPI formula and following the KDIGO eGFR category definition [1].

	PTH	Phosphate	25OH vitamin D	FGF23
G1 eGFR≥90 ml/min/1.73m2				
PTH	---	-0.112	-0.300	-0.034
		ns	(p <0.0001)	ns
FGF23	-0.034	0.1041	-0.1583	---
	ns	ns	ns	
CKD-EPI eGFR	-0.164	-0.0205	-0.015	-0.0373
	(p 0.0326)	ns	ns	ns
G2 eGFR 60-89 ml/min/1.73 m^2				
PTH	---	-0.097	-0.3325	0.1449
		ns	(p<0.0001)	(p 0.0085)
FGF23	0.1449	0.1316	-0.1243	----
	(p 0.0085)	(p 0.0171)	(p 0.0241)	
CKD-EPI eGFR	-0.0833	-0.0458	-0.0146	-0.1808
	ns	ns	ns	(p 0.0009)
G3-G5 eGFR<60 ml/min/1.73 m^2				
PTH	---	-0.202	-0.318	0.270
		ns	(p 0.001)	(p 0.006)
FGF23	0.270	0.205	-0.024	---
	(p 0.006)	ns	ns	
CKD-EPI eGFR	-0.559	0.052	-0.090	-0.342
	(p 0.000)	ns	ns	(p 0.000)
All				
PTH	---	-0.0918	-0.3229	0.2012
		(p 0.0252)	(p<0.0001)	(p<0.0001)
FGF23	0.2012	0.1173	-0.1369	---
	(p<0.0001)	(p 0.0042)	(p 0.0008)	
CKD-EPI eGFR	-0.2874	-0.0154	0.0097	-0.3425
	(p<0.0001)	ns	ns	(p<0.0001)

Ns: non-significant difference

Table 3

A. Multivariate analysis of predictors of PTH levels

	r	95 % CI		
		Low	High	P
Constant	4.700	4.187	5.214	**<0.0001**
Age	0.000	-0.003	-0.003	0.8997
Sex (male)	-0.084	-0.155	-0.012	**0.0215**
Diabetes	-0.145	-0.212	-0.077	**<0.0001**
Hypertension	0.053	-0.012	0.118	0.1111
Current Smoker	-0.086	-0.202	0.029	0.1421
Dyslipidemia	-0.051	-0.109	0.007	0.0858
Ejection fraction <40%	0.050	-0.039	0.139	0.2666
eGFR (CKD-EPI)	-0.006	-0.008	-0.004	**<0.0001**
HS C-reactive protein	-0.058	-0.114	-0.002	**0.0419**
Calcidiol	-0.014	-0.017	-0.010	**<0.0001**
Phosphate	-0.068	-0.122	-0.015	**0.0119**
Ln FGF 23	0.084	0.031	0.137	**0.0019**
Body Mass Index	0.003	-0.003	0.010	0.3460
Cerebrovascular events	-0.022	-0.165	0.121	0.7642
Peripheral artery disease	0.031	-0.110	0.172	0.6665
Atrial Fibrillation	-0.026	-0.165	0.113	0.7117

R^2= 0.2747

B. Multivariate analysis of predictors of FGF-23 levels

	r	95 % CI		P
		Low	High	
Constant	4.212	3.274	5.150	**<0.0001**
Age	-0.005	-0.010	-0.000	**0.0407**
Sex (male)	-0.191	-0.301	-0.080	**0.0007**
Diabetes	0.048	-0.058	0.155	0.3739
Hypertension	0.034	-0.067	0.135	0.5033
Current Smoker	0.077	-0.102	0.257	0.3969
Dyslipidemia	-0.029	-0.119	0.062	0.5310
Ejection fraction <40%	0.029	-0.109	0.167	0.6783
eGFR (CKD-EPI)	-0.009	-0.012	-0.006	**<0.0001**
HS C-reactive protein	0.106	0.019	0.193	**0.0165**
Calcidiol	-0.002	-0.008	0.003	0.3895
Phosphate	0.101	0.018	0.183	**0.0171**
Ln PTH	0.202	0.075	0.330	**0.0019**
Body Mass Index	0.002	-0.008	0.013	0.6465
Cerebrovascular events	-0.081	-0.303	0.141	0.4730
Peripheral artery disease	0.202	-0.016	0.420	0.0694
Atrial Fibrillation	0.199	-0.016	0.415	0.0692

R^2= 0.178

CI: Confidence Interval; eGFR: estimated Glomerular Filtration Rate; HS: High Sensitivity; FGF-23: Fibroblast Growth Factor-23. PTH: Parathormone.

Table 4. Estimated glomerular filtration rate and parameters of mineral metabolism stratified by age

	≤ 60 years **n=358**	**>60-75 years** **n = 243**	**>75 years** **n=103**	**p**
eGFR (ml/min/1.73m²)	85.1 ± 16.5	69.6 ± 16.2	58.5 ± 16.0	**<0.001**
Calcidiol (ng/dl)	19.2 (14.1 - 24.5)	19.3 (13.5 - 25.6)	16.1 (12.8 - 21.3)	**0.015**
FGF23 (RU/dl)	66.6 (51.7 - 85.0)	76.5 (56.8 - 103.8)	81.3 (61.1 - 124.9)	**<0.001**
PTH (pg/dl)	55.5 (43.0 - 69.6)	60.2 (46.9 - 80.8)	77.6 (50.8 - 97.9)	**<0.001**
Phosphate (mg/dl)	3.22 ± 0.57	3.17 ± 0.53	3.23 ± 0.53	0.484

Categorical variables are presented as percentages, quantitative variables with normal distribution as mean±SD and those not normally distributed as median (interquartile range).

eGFR: estimated Glomerular Filtration Rate; **FGF-23:** Fibroblast Growth Factor-23¸ **PTH:** parathormone.

FIGURE LEGEND

Figure 1. CKD-MBD parameters in different estimated glomerular filtration rate (eGFR) categories (G1 Normal renal function, eGFR > 90 ml/min/1.73 m^2, G2 Mildly decreased eGFR 60-89 ml/min/1.73 m^2, and G3-5 < 60 ml/min/1.73 m^2). A. Serum PTH; B. Serum FGF23; C. Serum 25 OH vitamin D; D. Serum phosphate.

Figure 2. Correlation between PTH, FGF23, 25 OH vitamin D or serum phosphate and estimated glomerular filtration rate (eGFR)