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Association of bone turnover markers and arterial stiffness in pre-dialysis chronic kidney disease (CKD)

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ABSTRACT

Vascular calcification (VC) is highly prevalent in CKD and leads to increased vascular stiffness and cardiovascular disease (CVD). Non-traditional cardiovascular risk factors include abnormal bone turnover and/or dysregulation of the calcification inhibitors, although their relative contribution remains unclear. We investigated the association between bone turnover, the calcification inhibitors (matrix gla protein; MGP and Fetuin-A), and the phosphate regulating hormone; fibroblast growth factor-23 (FGF-23) and arterial stiffness in pre-dialysis CKD patients. One hundred and forty-five patients with CKD stages 1–4 (74 M, 71 F) aged (mean [SD]) 53 [14] years were studied. Bone turnover markers (bone-specific alkaline phosphatase (BALP) and tartrate-resistant acid phosphatase (TRACP)) and MGP, Fetuin-A and FGF-23 were determined. BMD was measured at the lumbar spine (LS), femoral neck (FN), forearm (FARM) and total hip (TH). Arterial stiffness was assessed by contour analysis of digital volume pulse (SI_{DVP}). There was a significant positive correlation between TRACP:BALP ratio and SI_{DVP} ($r = 0.19$, $p = 0.023$). Following multi-linear regression analysis, significant associations were seen between serum BALP ($p = 0.037$), TRACP ($p = 0.009$) and TRACP:BALP ratio ($p = 0.001$) and SI_{DVP} independently of traditional CVD risk factors. No significant relationship between SI_{DVP} and MGP, Fetuin-A and FGF-23 was observed. A significant negative correlation was seen between BMD at the FARM and SI_{DVP} in CKD stage 4 ($r = -0.35$, $p = 0.024$). The association remained significant following correction for age, gender and cardiovascular risk factors ($p = 0.029$). Our data suggest a link between imbalances in bone turnover and arterial stiffness in pre-dialysis CKD. Longitudinal studies are needed to evaluate the clinical usefulness of these bone turnover markers as predictors of CVD in CKD.

Introduction

Chronic kidney disease (CKD) is associated with increased cardiovascular morbidity and mortality [1]. Vascular calcification (VC) is a common finding in CKD and has been shown to contribute to the high prevalence of cardiovascular disease in this population [2]. VC leads to stiffening of the larger, compliance-providing, arteries which has important clinical consequences including raised systolic blood pressure, left ventricular hypertrophy, reduced coronary perfusion and increased incidence of cardiovascular events and reduced patient survival [3]. The pathogenesis of VC is complex and involves several risk factors and mechanistic pathways, including traditional cardiovascular risk factors as well as non-traditional bone-related factors. One of the proposed aetiological factors in CKD is disturbances in calcium/phosphate homeostasis and abnormal bone turnover [4]. It is thought that the increases in bone resorption and/or reduction in bone formation which occur in CKD increases the availability of calcium and phosphate in the extracellular fluid and lead, in part, to excess deposition as hydroxyapatite crystals in the media of arterial walls. Indeed, several studies have shown an inverse correlation between bone mineral density (BMD) and VC in populations with imbalances in skeletal remodelling such as post-menopausal and elderly women with osteoporosis and patients with end stage kidney disease (ESRD) [5,6]. Another important process in the development and progression of VC is the dysregulation or loss of mineralisation inhibitors such as matrix gla protein (MGP) and Fetuin-A as they prevent the precipitation of calcium and phosphate in the arterial wall [7]. Animal studies have demonstrated that MGP and Fetuin-A inhibit calcification by forming a high molecular mass complex made up of calcium, phosphate, MGP and Fetuin-A [8]. Fibroblast growth factor −23 (FGF-23) has also been recently implicated as its synthesis is increased in CKD and it is an important

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regulator of phosphate homeostasis [9]. Furthermore FGF-23 has been shown to affect bone mineralisation and is independently associated with aortic calcification in ESRD [10,11].

The relative importance and contribution of abnormalities in bone turnover, loss of calcification inhibitors and increased FGF-23 in the aetiology of VC in pre-dialysis CKD remain unclear and need further evaluation. The aim of this study was therefore to investigate the association between bone turnover, the calcification inhibitors (MGP and Fetuin-A), circulating FGF-23 and arterial stiffness in pre-dialysis CKD patients. Recognition of the major factors which determine arterial stiffness in the pre-dialysis stage of CKD will make assessment and targeted therapeutic intervention possible at an early stage.

Subjects and methods

Subjects

One hundred and forty-five ambulant patients (74 M, 71 F) aged (mean [SD]) 53 [14] years attending the renal unit with CKD stages 1–4 were included in the study. Ethical approval was obtained from the Research and Ethics Committee of Guy's and St. Thomas' hospitals. They were recruited from consecutive weekly renal out-patient clinics over a period of 9 months. Patients with previous history of parathyroidectomy or recent hospital admissions (<1 month), on renal replacement therapy or on treatment with active vitamin D sterols, phosphate binders or bisphosphonates were excluded from the study as described previously [12]. The patients were divided into three groups based on their estimated GFR (eGFR)—Group 1: eGFR >60 mL/min; CKD 1 and 2 (n = 41); Group 2: eGFR 30–59 mL/min; CKD 3 (n = 59); Group 3: eGFR <30 mL/min, CKD 4. (n = 45). Demographics and clinical details including body mass index (BMI), waist circumference (WC), blood pressure and co-morbidities were obtained from a questionnaire which was applied to all the participants (Table 1). Following informed consent, non-fasting blood and spot urine samples were collected. Routine biochemical analyses were carried out immediately and additional serum and plasma samples were frozen at −70 °C for analysis of FGF-23, bone-specific alkaline phosphatase (BALP), tartrate-resistant acid phosphatase (TRACP-5b), matrix gla protein (MGP), Fetuin-A and uncarboxylated factor (PIVKA-II), a sensitive functional marker of vitamin K status and a specific marker of the γ-carboxyglutamate status of coagulation factor II synthesised in the liver. Vitamin K is an essential cofactor for the synthesis of γ-carboxyglutamate residues that in MGP play important roles in mineral and protein binding abilities of MGP. Digital volume pulse was measured in the renal clinic and a DXA scan to assess BMD was requested on all study participants.

Table 1
Summary of patient demographics and clinical details.

Characteristic	CKD 1, 2	CKD 3	CKD 4
eGFR mL/min (Mean ± SD)	78 ± 14	45 ± 8	21 ± 4
Number	41	59	45
Age (yr)	47 ± 13	57 ± 15	55 ± 13
Female (%) (n)	56 (23)	37 (22)	58 (26)
Black race (%) (n)	19 (8)	29 (17)	20 (9)
Body mass index (kg/m^2)	28 ± 7	29 ± 5	27 ± 6
Smokers % (n)	22 (9)	17 (10)	13 (6)
Blood pressure (mm Hg)			
Systolic (Mean ± SD)	127 ± 25	129 ± 17	133 ± 23
Diastolic (Mean ± SD)	74 ± 12	76 ± 11	78 ± 14
Aetiology of CKD % (n)			
Diabetes	2.4 (1)	15.3 (9)	17.8 (8)
Hypertension	9.8 (4)	27.1 (16)	22.2 (10)
Glomerular disease	9.8 (4)	5.1 (3)	11.1 (5)
Renovascular disease	7.3 (3)	8.5 (5)	8.9 (4)
Polycystic kidney disease	17.1 (7)	10.2 (6)	13.3 (6)
IgA nephropathy	14.6 (6)	8.5 (5)	4.4 (2)
Reflux nephropathy	7.3 (3)	3.4 (2)	8.9 (4)
Other causes	17.1 (7)	23.7 (14)	24.4 (11)
Unknown cause	12.2 (5)	6.8 (4)	4.4 (2)
Duration of CKD (years)	7 (0.2–50)	4 (0.2–36)	7 (0.3–60)
Median (range)			
Co morbidities % (n)			
Hypertension	73.2 (30)	79.7 (47)	93.3 (42)
Diabetes	12.2 (5)	18.6 (11)	17.8 (8)
CVD	9.8 (4)	16.9 (10)	8.9 (4)
PVD	7.3 (3)	10.2 (6)	13.3 (6)
Stroke	7.3 (3)	8.5 (5)	4.4 (2)

CKD—chronic kidney disease, CVD—cardiovascular disease, PVD—peripheral vascular disease.

Laboratory measurements

Serum creatinine, calcium, phosphate, albumin, bilirubin, liver enzymes, lipid profile and urinary phosphate and protein creatinine ratios were measured by standard laboratory methods using Roche Modular analysers (Roche diagnostics Limited, West Sussex RH15 9RY, UK). Measurement of high sensitivity CRP was done by particle enhanced immunonephelometry (Dade Behring Inc. Newark, DE 19714 U.S.A.) on BN ProSpec analyser. Serum intact PTH was measured by an electrochemiluminescence immunoassay on the Roche Elecsys 2010 analyser (Roche Diagnostics, Indianapolis) and 25-hydroxyvitamin D (25(OH) vitamin D) by Radioimmunoassay (RIA) (Diasorin Inc, Stillwater, MN, USA). Inter-assay CV was 7.9% and 7.3% at serum 25(OH)vitamin D of 40.4 nmol/L and 131.8 nmol/L respectively. 1,25 dihydroxyvitamin D was measured by Radioimmunoassay (IDS, Boldon, UK) as previously described [12] . Measurement of Plasma FGF-23 was carried out by a two-site enzyme-linked immunosorbent assay (ELISA) (Immutopics, Inc. San Clemente CA 92673) which detects both the intact molecules and the C-terminal fragments. Intra-assay CV was 8.9% and 8.6% and inter-assay CV was 7.7% and 8.3% at serum FGF-23 concentrations of 37 RU/L and 370 RU/L respectively. TRACP and BALP were measured by ELISA and immunoenzymetric assay respectively (Immunodiagnostics systems limited, Boldon, Tyne and Wear, NE35 9PD) with an inter-assay CV of 9.7% and 4.7% at concentrations 2.5 U/L and 7.5 U/L respectively for TRACP and 1.9% and 9.5% at concentrations 10.6 μg/L and 42.7 μg/L respectively for BALP. TRACP: BALP ratio was derived as a marker of imbalance between bone resorption and formation rate. MGP was measured by competitive enzyme immunoassay (Biomedica Medicine Product GmbH & Co KG, A-1210 Vienna) and Fetuin-A was measured by sandwich enzyme immunoassay (BioVendor GmbH, D-69120 Heidelberg, Germany). The inter-assay and intra-assay CV for MGP was 12.8% and 2.3% respectively at serum MGP of 6.2 nmol/L. Inter-assay and intra-assay CV for Fetuin-A was 7.7% and 7.5% respectively at serum Fetuin-A of 11.6 ng/mL. Serum PIVKA-II was determined using a MAb (C4B6) to PIVKA-II in an ELISA format. The C4B6 MAb used in this assay is conformation-specific such that in the presence of calcium ions it binds undercarboxylated species of prothrombin and does not cross-react with fully-carboxylated native prothrombin [13]. Results are expressed as arbitrary units (AU) per millilitre, where 1 AU is equivalent to 1 μg of multiple PIVKA-II species. Using this assay the upper limit of the reference range and the lower limit of detection for PIVKA-II in adults was <0.2 AU/mL (200 ng/mL). The inter-assay coefficient of variation was <10%.

Bone mineral Density (BMD)

BMD at the total hip (TH), femoral neck (FN), lumbar spine (LS) and forearm (FARM) were measured using DXA scan (Discovery A QDR Series, Hologic, Inc. USA). The CV for BMD measurement was 0.35%.

Arterial stiffness index (SI)

Arterial stiffness was assessed by contour analysis of digital volume pulse (DVP) which provides an index of large artery stiffness. DVP was measured by passing an infrared light through the finger pulp which produces a typical waveform made up of a direct component and a reflected component. The direct or systolic component is produced by the pressure wave transmitted directly from the left ventricle to the finger and the reflected or diastolic component is produced by the pressure wave transmitted along the aorta to small arteries in the lower limb from where it is reflected back along the aorta to the finger. The height of the individual divided by the time delay between the direct and reflected waves in the DVP gives the stiffness index (SI). SI derived from the analysis of digital volume pulse (SI_{DVP}) has been shown to provide a measure of stiffness similar to pulse wave velocity (PWV) which is normally determined by measuring carotid-femoral PWV (PWV_{cf}). Previous studies have shown good correlation with between SI_{DVP} and PWV_{cf} in asymptomatic subjects ($r=0.65$, $p<0.0001$) [14] and in patients with ESRD ($r=0.66$, $p<0.001$) [15]. The variation of SI_{DVP} with age and blood pressure was similar to that seen with PWV_{cf} [14]. SI_{DVP} has also been found to be associated with cardiovascular risk scores and useful in identifying high risk population [16]. SI_{DVP} was measured using the Pulse Trace System (Micro Medical Limited, Rochester, Kent, England ME1 2AZ). The SI_{DVP} was measured a minimum of 3 times on each study participant by the same investigator and the mean measurement was derived. The mean within-subject coefficient of variation (CV) was 8% and 5–10% at values of 4 m/s and 10.0 m/s respectively. Measurement of SI_{DVP} therefore provides a reproducible, non-invasive, simple and rapid method of assessing arterial stiffness.

Statistical analysis

Mean and standard deviation were calculated for all variables. Comparisons between groups were performed using Mann–Whitney *U* test. Pearson's correlation analysis was undertaken to determine the correlation between variables in univariate analyses. Multiple linear regression analysis was undertaken to explore the relationship of SI_{DVP} with bone turnover, the calcification inhibitors and FGF-23 adjusted for age, gender. Other potential confounding variables which were found to correlate significantly with SI_{DVP} following univariate analyses were included into the multi regression model. As several of the independent variables such as BALP and TRACP, diastolic and systolic blood pressure are known to be inter-related, a collinearity test was performed to derive the variance inflation factor (VIF) and only one regressor was included in the model if VIF was greater than 2. A '*p*' value of <0.05 was considered statistically significant. Statistical analyses was performed using the standard statistical software package, SPSS 17.0 for Windows (LEADTOOLS (c), LEAD technologies, Inc, US).

Results

Biochemical data of the study population

The biochemical parameters including the SI_{DVP} and BMD data in the study subjects with CKD stages 2–4 are summarised in Table 2. There was no significant difference in serum cholesterol between the CKD groups. TRACP and FGF-23 were significantly higher in CKD stages 3 and 4 ($p<0.05$) compared to subjects with CKD 1 and 2. BALP was higher in CKD stage 4 only. Serum MGP was significantly lower in CKD stage 4 compared to CKD 1/2 ($p<0.05$). There were 12 patients with abnormal PIVKA-II levels. Four of these patients had PIVKA-II levels>10 AU/mL—all four of these patients were chronically anticoagulated with warfarin. Eight non-anticoagulated patients had detectable PIVKA-II (>0.2 AU/mL). In these patients the mean [SD] PIVKA-II level was 0.54 [0.32] AU/mL consistent with low vitamin K tissue stores (or impaired vitamin K utilisation) and showing mild impairment of vitamin K-dependent protein carboxylation of factor II. Three patients had CKD stage 3 and 5 had CKD stage 4. PIVKA-II was not detected in any patients with CKD stage 2. Fetuin-A did not differ significantly between the groups. There was no significant difference in SI_{DVP} between the 3 CKD groups. In contrast, BMD measured in 119 patients and expressed as 'Z' scores was significantly lower at the FN and TH in CKD 4.

Table 2
Summary of the biochemical data, SI_{DVP} and BMD (expressed as 'Z' scores) of the study population. The values are expressed as Mean ± SD.

Analyte	CKD 1, 2 (n = 41)	CKD 3 (n = 59)	CKD 4 (n = 45)
eGFR (mL/min)	78 ± 14^{nn}	$45 \pm 8^{**}$	21 ± 4^{aa}
Albumin corrected calcium (mmol/L)	2.4 ± 0.10	$2.36 \pm 0.11^{*}$	2.31 ± 0.12
Serum phosphate (mmol/L)	1.12 ± 0.15	$1.19 \pm 0.20^{*}$	1.33 ± 0.31^{aa}
PTH (ng/L)	42.2 ± 24.7^{nn}	$67.0 \pm 40.4^{**}$	$130. \pm 112.0^{aa}$
1,25 $(OH)_2$ D_3 (pmol/L)	89 ± 41.5^{nn}	$67.5 \pm 32.1^{**}$	44 ± 34.6^{aa}
BAP (μg/L)	14.1 ± 7.9	$15.0 \pm 6.9^{*}$	19.7 ± 11.3^{a}
TRACP (U/L)	2.5 ± 0.8^{n}	3.0 ± 1.0	3.4 ± 1.2^{a}
hs-CRP (mg/L)	3.2 ± 5.8^{n}	4 ± 5.8	6.3 ± 15
FGF-23 (RU/L)	53 ± 54	97 ± 163	137 ± 192^{aa}
Total cholesterol (mmol/L)	4.7 ± 0.9	5.0 ± 1.0	4.6 ± 1.2
MGP (nmol/L)	35.3 ± 17.6	30.2 ± 8.2	27.6 ± 9.1^{a}
Fetuin-A (ng/mL)	28.9 ± 5.4	27.9 ± 5.3	28.2 ± 12.1
SI_{DVP} (m/s)	9.4 ± 2.1	10.2 ± 2.4	10.0 ± 2.5
'Z' score hip	0.42 ± 0.90	$0.64 \pm 1.28^{*}$	0.04 ± 1.04
'Z' score femur	0.30 ± 0.99	$0.33 \pm 1.25^{*}$	−0.16 ± 1.02
'Z' score spine	0.18 ± 1.21	$0.80 \pm 1.75^{*}$	0.07 ± 1.57
'Z' score forearm	0.20 ± 1.15	0.52 ± 1.43	−0.03 ± 1.31

^{n}CKD 1, 2 vs 3 P<0.05, ^{nn}CKD 1, 2 vs 3 P<0.001, *CKD 3 vs 4 P<0.05, **CKD 3 vs 4 P<0.001, ^{a}CKD 1, 2 vs 4 P<0.05, ^{aa}CKD 1, 2 vs 4 P<0.001.

Relationship between the variables and SI_{DVP}

In univariate analyses, SI_{DVP} correlated significantly with age ($r=0.17$, $p<0.05$) and with systolic blood pressure ($r=0.19$, $p<0.05$), diastolic blood pressure ($r=0.29$, $p<0.001$), total cholesterol ($r=0.23$, $p<0.01$) and LDL-Cholesterol ($r=0.22$, $p<0.02$). A significant correlation was also observed between SI_{DVP} and TRACP: BALP ratio ($r=0.19$, $p=0.023$) as illustrated in Fig. 1.

Multiple linear regression analysis with the bone turnover markers, calcification inhibitors, FGF-23 entered as independent variables after correction for age, gender, systolic blood pressure, total cholesterol, CRP, vitamin K status (as determined by PIVKA-II) showed that BALP and TRACP were the only significant determinants of SI_{DVP}. BALP (marker of bone formation) was negatively associated with SI_{DVP} ($p=0.037$). In contrast, TRACP (marker of bone resorption) was positively correlated with SI_{DVP} in multivariate analysis

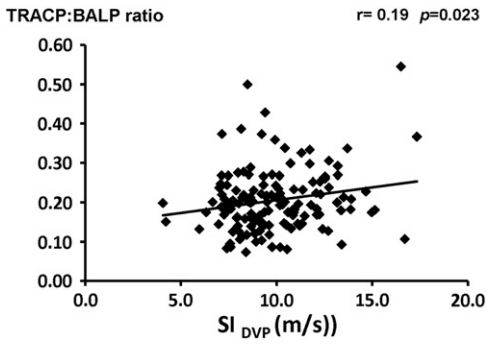

Fig. 1. Correlation between TRACP:BALP ratio and SI_{DVP}. Higher bone resorption and lower bone formation is associated with increases in SI_{DVP}.

Table 3
Multi-linear regression analysis of SI_{DVP} as dependent variable. The significant determinants are shown in bold.

Variables	β coefficients	t value	p value
Age	0.163	1.56	0.12
Gender	0.060	0.62	0.54
Systolic BP	0.14	1.3	0.19
Total cholesterol	**0.28**	**2.83**	**0.006**
CRP	0.09	0.88	0.38
FGF-23	−0.11	−1.1	.277
PIVKA11	0.001	0.005	0.996
MGP	−0.045	−0.45	0.65
Fetuin-A	−0.045	−0.46	0.65
Previous fracture	0.15	1.6	0.1
BALP	**−0.253**	**−2.12**	**0.037**
TRACP	**0.31**	**2.66**	**0.009**

$R^2 = 0.232$.

($p = 0.009$). No significant independent association was observed with MGP, Fetuin-A and FGF-23. The results are summarised in Table 3. The relationship between SI_{DVP} and the bone turnover markers became stronger when they were expressed as a ratio (TRACP: BALP) ($p = 0.001$) and entered in the same statistical model.

Although no significant association was observed between circulating MGP and Fetuin-A and SI_{DVP}, these 2 calcification inhibitors correlated significantly with systolic blood pressure (MGP: $r = 0.19$, $p < 0.05$, Fetuin-A: $r = -0.17$, $p < 0.05$) in univariate analyses. In addition, significant positive correlations were seen between MGP and BMI ($r = 0.21$, $p < 0.05$), eGFR ($r = 0.21$, $p < 0.05$) and serum calcium ($r = 0.18$, $p < 0.05$).

Relationship between BMD and SI_{DVP}

SI_{DVP} did not correlate significantly with BMD at any site in the whole study population. As BMD was lower in the group of patients with CKD 4, we performed sub-group analyses looking at the relationship between BMD and SI_{DVP} in CKD 4. Following univariate analysis, a significant negative correlation was seen between BMD at the FARM ($r = -0.35$, $p = 0.024$) the lower the BMD, the higher the SI_{DVP} (Fig. 2). After adjustment made for age, gender, systolic blood pressure, and cholesterol, in multivariate analysis, the significant association between SI_{DVP} and BMD at the FARM persisted (Table 4). This model explained 40% of the variance in SI_{DVP} in CKD stage 4.

Discussion

Vascular calcification (VC) is highly prevalent in CKD and affects as many as 40% of patients with GFR <33 mL/min [1]. VC results in arterial stiffness which contributes to the increased cardiovascular events observed in this population. Several traditional risk factors such as age, male gender, dyslipidaemia, hypertension and chronic

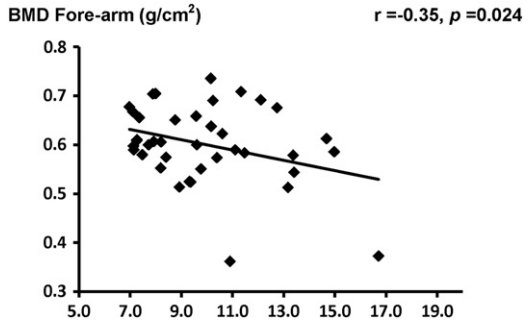

Fig. 2. Correlation between SI_{DVP} and BMD at the forearm in CKD stage 4. Increases in SI_{DVP} is linked with lower BMD.

Table 4
Multi-linear regression analysis of SI_{DVP} as dependent variable in CKD stage 4 with BMD at the forearm as an independent determinant. The significant variables are shown in bold.

Variables	β coefficients	t value	p value
Age	0.277	1.936	0.06
Gender	**0.29**	**2.04**	**0.049**
SBP	.272	1.95	0.059
TC	0.239	1.67	0.1
BMD forearm	**−0.33**	**−2.28**	**0.029**

$R^2 = 0.4$.

inflammation are promoters of VC. There are however several non-traditional factors which are specific to CKD which also contribute to increased VC [1]. This study was designed to evaluate the contribution of the different mechanistic pathways which might lead to increased arterial stiffness in CKD namely the abnormalities of bone metabolism as determined by humoral markers of bone turnover (BALP, TRACP-5b) and calcification inhibitors (MGP, Fetuin-A) and FGF-23.

Our study demonstrates that the bone turnover markers were associated with arterial stiffness, independently of the traditional risk factors. Disorders of skeletal remodelling and mineral metabolism in CKD, termed chronic kidney disease mineral bone disorder (CKD-MBD), has been directly linked with excess cardiovascular mortality in CKD [4]. Both ends of the spectrum of bone turnover abnormalities in CKD seem to play an important role in its pathophysiology. High turnover rate bone disease in CKD, caused by secondary hyperparathyroidism, stimulates bone resorption and results in the increased release of calcium and phosphate in the extracellular fluid [17]. In the low bone turnover bone disease (adynamic bone disease, ABD), decreased bone formation results in failure of skeletal incorporation of calcium and phosphate or a reduction in the buffering ability of extracellular calcium and phosphate leading to fluctuations and disordered homeostasis [17]. Both situations lead to a state of positive phosphate balance which provides the stimulus for heterotopic calcification. However the correlation between serum levels of calcium and phosphate and vascular calcification has not been consistent [18,19]. Single or small numbers of serum phosphate values may not be sensitive enough to reflect the varying concentrations in the vascular milieu, 85–90% of phosphate is intra- not extracellular [18]. Assessment of skeletal remodelling which is one of the main pathophysiological mechanisms affecting mineral homeostasis may be a better predictor of vascular calcification and arterial stiffness in CKD. In our study we found a significant association between higher TRACP and lower BALP concentrations and increased arterial stiffness, suggesting that the imbalance between bone resorption and formation in CKD as indicated by the TRACP:BALP ratio is highly predictive of vascular stiffness. The association between cardiovascular events and the bone turnover markers; BALP and TRACP has been previously reported in CKD [20]. In contrast to our findings however, the authors found that the bone formation marker; BALP was positively associated with cardiovascular events whereas lower TRACP was predictive of the same clinical events. A positive correlation has also been described between BALP and carotid internal diameter in CKD, although the authors did not find a significant association with carotid stiffness and compliance [21]. The discrepant observations may be due, in part, to the differences in study design as patients with CKD stage 5 were included in these studies and different outcome measures were analysed [20,21]. Another explanation, particularly of the positive association between cardiovascular outcomes and BALP, may be due to increased production of BALP-type alkaline phosphatase from the calcified vascular lesions, a finding which may not be evident in our study population as we did not include patients with advanced CKD (CKD stage 5). In support of our data, a recent study in patients with earlier stages of CKD suggest that decreased bone formation as measured by bone histomorphometry, is

independently associated with increased coronary artery calcification and thus provides further evidence that low bone formation rate is another non-traditional risk factor for cardiovascular disease in pre-dialysis CKD [22].

Several epidemiological studies have shown that a reduction in bone mass or BMD in the elderly and in women with post-menopausal osteoporosis is associated with increased VC and CVD [23]. Lower BMD in CKD and ESRD has also been associated with increased cardiovascular morbidity and mortality in some studies [24,25]. Measurement of the forearm has been shown to be the best predictor of fracture risk in the population with primary renal disease [26]. We found an independent correlation between arterial stiffness and BMD at the forearm in patients with CKD stage 4 only. This suggests that the relationship between the changes in BMD and arterial stiffness are seen mainly when GFR is less than 30 mL/min. This would indicate that changes in the bone turnover markers may provide earlier information of cardiovascular risk than BMD. Nevertheless the independent association between arterial stiffness and BMD provides further support to the hypothesis of a pathophysiological link between skeletal and vascular metabolism in CKD.

The other mechanism which promotes VC in CKD is loss of physiological inhibitors of calcification or their activity. A number of these proteins are present in the circulation and changes in their circulating concentrations or functionality may be associated with arterial stiffness. These include MGP and Fetuin-A. Knock out of the MGP and Fetuin-A genes lead to arterial and soft tissue calcification under appropriate conditions [27]. High expression of MGP has been demonstrated in the vascular tissue near atherosclerotic plaques and has been linked to calcification of vascular smooth muscle cells [28]. Vitamin-K dependent gamma-carboxylation is required for its biological activity. Circulating concentrations of MGP has been shown to be negatively associated with coronary artery calcification and plasma concentrations of the inactive uncarboxylated form are lower in CKD and ESRD [29,30]. However, this finding has not been universally confirmed as the inactive non-phosphorylated and uncarboxylated and total MGP have been shown to be higher in ESRD and patients with severe atherosclerosis in some studies [31–33]. This may be explained partly by assay differences and measurement of different forms of MGP as the measurement of MGP is complicated by the fact that the protein undergoes two independent post-translational modifications (γ-glutamyl carboxylation and phosphorylation) and by the limitations of current assays for distinguishing between these forms [34,35]. For the present study the only available commercial MGP assay was one which measures total non-phosphorylated MGP and which does not discriminate between carboxylated and uncarboxylated forms [34,35]. In our study, we found lower levels of total non-phosphorylated MGP in patients with CKD stage 4. One possible explanation is a reduction in the uncarboxylated sub-fraction due to sequestration in the calcified arterial wall as a result of its affinity for calcium. Indeed, we observed a significant correlation between serum calcium and total MGP. A limitation of our study is that we could not measure the inactive non-phosphorylated, undercarboxylated fraction separately and this was recently shown to be a surrogate marker for vascular calcification in CKD [32]. Another limitation is that PIVKA-II may not accurately reflect the function of vitamin K-dependent MGP on vascular tissues. A significant correlation was seen with systolic blood pressure and MGP, although we did not see any significant association between MGP and arterial stiffness. This finding can be explained, at least in part, by confounding by other traditional cardiovascular risk factors and/or the limitations of the assay used to measure MGP. The association between total MGP and coronary risk factors including systolic blood pressure despite an inconsistent association with arterial calcification has been previously documented in community-based cohorts [36] and suggest that circulating total MGP may be a weak predictor of arterial stiffness, at least in early CKD.

One proposal is that MGP exerts its effect on the inhibition of calcification through the formation of a stable complex with Fetuin-A which binds mineral including calcium and phosphate, thus preventing the formation of calcium hydroxyapatite crystal [8]. Low serum Fetuin-A in CKD and ESRD has been associated with accelerated vascular calcification and increased cardiovascular events, although the relationship has been more consistent in ESRD cohorts where the clinical picture and biochemical abnormalities are more severe and the prevalence of CVD is higher [37]. In our study of pre-dialysis patients, we did not see any changes in circulating concentrations of Fetuin-A with worsening renal impairment or any relationship with vascular stiffness or the inflammatory marker; C-reactive protein. However, a negative correlation was observed with systolic blood pressure, although the clinical significance of this association remains to be determined. Further clarification of the role of Fetuin-A in CKD is required.

It is now established that FGF-23 is a regulator of phosphate homeostasis. Circulating concentrations of FGF-23 is high in CKD and ESRD [9]. Several studies have shown that elevated FGF-23 is associated with abnormal bone metabolism in renal disease [12,38]. In addition recent studies have suggested that FGF-23 has 'off-target' effects, particularly in ESRD [9]. High FGF-23 concentrations have been related to increased vascular calcification, left ventricular hypertrophy and increased cardiovascular mortality in ESRD and in patients with CVD [9,39,40]. Thus it has been postulated that FGF-23 may provide a biomarker that predicts, not only bone and mineral abnormalities but also vascular calcification and mortality, particularly in ESRD [11]. FGF-23 was not significantly associated with vascular stiffness in this study and may be due, in part, to the degree of renal impairment in our study population. In early or pre-dialysis CKD, FGF-23 increases in an attempt to maintain phosphate homeostasis. When this attempt at regulation fails in CKD stage 5 or ESRD, the relationship between elevated FGF-23 and arterial calcification may become stronger. It is also possible that the effect of FGF-23 on the cardiovascular system in CKD may be related, at least partly, to its effect on the myocardium [41] or via other mechanisms, independently of vascular calcification. We have previously shown a significant relationship between FGF-23 and the inflammatory marker; CRP which is another independent marker of vascular complications in CKD [12]. Whether FGF-23 is directly and actively implicated in the pathogenesis of CKD-MBD or merely a biomarker which provides information of mineral, skeletal and vascular abnormalities in CKD and ESRD remains to be established.

In summary, the main finding of this study is that bone turnover markers which reflect bone resorption and formation were independently associated with arterial stiffness in CKD. Increased vascular stiffness was associated with lower BMD at the forearm in CKD stage 4. Although the cross-sectional nature of the study limits the establishment of a causal relationship and the direction of the association between skeletal abnormalities and vascular stiffness, the data suggest that analysis of these bone turnover markers in CKD may provide additional information on cardiovascular risk.

In conclusion, our findings lend further support of the link between abnormalities in bone turnover and vascular stiffness and increased cardiovascular events. Longitudinal investigations should be undertaken to assess the usefulness of these markers in CKD over time and their predictive value in cardiovascular disease. The maintenance of normal bone turnover may be a very important clinical 'target' in the prevention of cardiovascular disease in CKD.

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