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ORIGINAL ARTICLE

Tumor necrosis factor-alpha is associated with mineral bone disorder and growth impairment in children with chronic kidney disease

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Abstract

Background Mineral and bone disorder (MBD) and growth impairment are common complications of pediatric chronic kidney disease (CKD). Chronic inflammation detrimentally affects bone health and statural growth in non-CKD settings, but the impact of inflammation on CKD-MBD and growth in pediatric CKD remains poorly understood. This study assessed associations between inflammatory cytokines with biomarkers of CKD-MBD and statural growth in pediatric CKD.

Methods This is a cross-sectional study of children with predialysis CKD stages II–V. Cytokines (IL-1b, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, interferon-γ), bone alkaline phosphatase (BAP), and procollagen type 1 N-terminal propeptide (P1NP) were measured at the same time as standard CKD-MBD biomarkers. Associations between cytokines, CKD-MBD biomarkers, and height *z*-score were assessed using linear regression analysis.

Results Among 63 children, 52.4% had stage 3 CKD, 76.2% non-glomerular CKD etiology, and 21% short stature. TNF-α was the only cytokine associated with parathyroid hormone (PTH) independent of glomerular filtration rate. After stratification by low, medium, and high TNF-α tertiles, significant differences in PTH, serum phosphorus, alkaline phosphatase, BAP, P1NP, and height *z*-score were found. In a multivariate analysis, TNF-α positively associated with phosphorus, PTH, and alkaline phosphatase and inversely associated with height *z*-score, independent of kidney function, age, sex, and active vitamin D analogue use.

Conclusions TNF-α is positively associated with biomarkers of CKD-MBD and inversely associated with height *z*-score, indicating that inflammation likely contributes to the development of CKD-MBD and growth impairment in pediatric CKD. Prospective studies to definitively assess causative effects of inflammation on bone health and growth in children with CKD are warranted.

Keywords Chronic kidney disease · CKD-mineral and bone disorder · Inflammation · TNF-α · Pediatric

Introduction

Despite recent advances in the field of chronic kidney disease (CKD), the prevalence of pediatric stage 5 chronic kidney disease (CKD 5) in the USA has been increasing [1]. CKD-mineral and bone disorder (MBD), a complication of CKD [2, 3], manifests by increases in circulating parathyroid hormone (PTH), phosphorus, alkaline phosphatase (ALP), fibroblast growth factor 23 (FGF23), and other biomarkers and leads to impaired bone health and vascular calcifications. In the chronic kidney disease in children (CKiD) study cohort, 67% of participants had CKD-MBD [4]. CKD-MBD increases the risk of fractures [5] and is associated with cardiovascular disease [6, 7]. In children, CKD-MBD contributes to the impairment of statural growth [8, 9]. In the North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS), 36.9% of children had short stature at enrollment [10].

Chronic low-grade inflammation is another typical feature of CKD [11]. Specifically, it has been shown that tumor necrosis factor-alpha (TNF-α) and IL-6 are inversely and independently associated with GFR in CKD [12]. The specific role

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of individual cytokines in CKD remains incompletely understood. We have recently performed cytokine profiling to identify cytokines associated with anemia in children with CKD [13]. Only IL-6, but not the other cytokines that we screened, was associated with anemia. Interestingly, in our subsequent experimental study, *IL-6* deletion, while improving anemia, failed to improve bone health, growth, and nutritional status of juvenile mice with CKD [13]. Thus, understanding of the role of individual cytokines in CKD pathophysiology is important and may inform novel therapeutic targets.

Chronic non-renal inflammatory disorders of childhood, such as juvenile idiopathic arthritis and inflammatory bowel disease, frequently present with bone disease [14–17] and poor statural growth [18, 19]. The use of TNF-α inhibitors in children with these conditions improves their bone health and growth [20, 21]. While it remains unclear if inflammation contributes to the development of CKD-MBD in children with CKD, emerging literature suggests that this is highly plausible. Indeed, serum phosphorus is independently associated with inflammation in adults with CKD [22]. Inflammation stimulates production of FGF23 [23]. In a population-based cohort, TNF-α was the only cytokine independently and positively associated with FGF23 [24]. Based on bone biopsy data, TNF-α was shown to inversely correlate with bone volume in adults with CKD [25]. TNF-α suppresses bone formation, particularly by inhibiting osteoblastic differentiation of mesenchymal stem cells in the bone [26]. Thus, based on the recent literature, investigation of the role of inflammation and specifically TNF-α in pediatric CKD-MBD and growth impairment is warranted.

The aim of the present study was to evaluate a panel of cytokines to detect potential inflammatory mediators implicated in CKD-MBD and in growth impairment of children with CKD.

Methods

Patients

This was a cross-sectional study. Children were enrolled from the pediatric nephrology clinics at Weill Cornell Medical Center from June 2016 to June 2019. The study was approved by the Institutional Review Board of Weill Cornell Medicine. Parental informed consent was obtained, as well as assent for children ≥ 7 years of age. Eligibility criteria included the following: pre-dialysis CKD stage II and above using the KDIGO definition [27] and no acute illnesses at the time of blood collection. Glomerular filtration rate (GFR) was calculated using the bedside Schwartz formula [28]. Age-sex-specific height *z*-scores were calculated using the 2000 Centers for Disease Control and Prevention standard growth charts for US children [29].

Biomarkers

Serum was separated immediately upon blood collection and stored at − 80 °C until analyzed. To delineate the role of individual cytokines in the development of CKD-MBD, we measured serum levels of 9 cytokines previously reported to be altered in CKD (IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, and interferon-γ). Cytokines were measured using V-PLEX kit, an array-based multiplex electrochemiluminescent assay, from Meso Scale Discovery (Rockville, MD) on the MESO QuickPlex SQ120 Analyzer following the manufacturer's instruction. The intra-assay coefficient of variation (CV) for these cytokines ranged from 2.4 to 6.4% and inter-assay CVs from 5.0 to 10.2%. The detection limits of IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, TNF-α, and interferon-γ were 0.10, 0.016, 0.10, 0.07, 0.077, 0.10, 0.50, 0.20, and 0.40 pg/mL, respectively. Serum bone-specific alkaline phosphatase (BAP) was measured by an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's manual (Quidel/Metra Bio Systems, San Diego, CA). The intra-assay and inter-assay CVs were < 6.5% and < 10.0%, respectively. The sensitivity was 0.7 μg/L. Serum procollagen type 1 N-terminal propeptide (P1NP) was measured by a radioimmunoassay following the manufacturer's instruction (Orion Diagnostica, Oulunsalo, Finland). The intra-assay and inter-assay CVs were < 10.2% and < 9.8%, respectively. The sensitivity was 2.0 μg/L. Serum phosphorus, PTH, and ALP were measured by the New York Presbyterian Hospital clinical laboratory as part of the standard of care.

Power considerations

Mendoza et al. reported a direct correlation between TNF-α and FGF23 with a coefficient of correlation $r = 0.4$ in adult patients with CKD from the Chronic Renal Insufficiency Cohort (CRIC) study [23]. Assuming a correlation with similar strength exists between TNF-α and PTH in children with CKD, a priori power analysis indicated that a total sample size of 47 patients will be required to determine the significance of correlation coefficient with type I error rate $\alpha = 0.05$ and type II error rate $\beta = 0.2$ [30].

Statistical analysis

The χ^2 test was applied to detect differences between categorical variables. Normality of continuous variables was checked using Shapiro-Wilk W test. To examine the relationship between TNF-α and clinical characteristics of CKD in children, we stratified our cohort by TNF-α tertiles. ANOVA was used to compare normally distributed continuous variables among TNF-α tertiles and Kruskal-Wallis test for non-normally

distributed variables. The correlations were assessed using Spearman's rank test. Multiple linear regression was used for multivariate analyses. To assess the relationship between TNF-α and CKD-MBD, and between TNF-α and growth, we conducted a series of stepwise linear regression analyses where TNF-α was the exposure. PTH, ALP, serum phosphorus, BAP, P1NP, and height *z*-score were the outcomes for the respective individual analyses. We first performed unadjusted analyses (model 1), followed by adjustment for age and sex (model 2), GFR (model 3), and active vitamin D analog use (model 4). *P* values < 0.05 were considered statistically significant. STATA 12.0 (Stata Corp, College Station, TX) was used for conducting statistical analyses.

Results

Cohort characteristics

The cohort was composed of 63 children with median GFR 42.0 [30.11–53.41] mL/min/1.73 m^2 (Table 1). Most children had stage III CKD. The majority had non-glomerular CKD etiology, predominantly congenital anomalies of the kidneys and urinary tract. Approximately half of the cohort had microalbuminuria. Although most children had normal body mass index (BMI), median height *z*-score was − 1.01 [− 2.1–0.04], and 21% of children had a short stature defined as height < 3rd percentile.

Median PTH was 98 [54–295] ng/L, and one-third of the children (33.3%) were receiving active vitamin D analogs. Nutritional vitamin D was prescribed to 34.9% of children, and median 25(OH)D level was 27.9 [19.9–32.0] ng/mL. Serum phosphorus was normal in the majority of children. Very few children required phosphate binders in this cohort. Alkali therapy was used in 14 children (22.2%) and recombinant human growth hormone (rhGH) in 11 children (17.4%). Children receiving rhGH at the time of blood sample collection for this study did not have statistically significant differences from the rest of the cohort, with the exception of sex and height *z*-score (Table S1).

Cytokine profiling identified an association between TNF-α and PTH

In unadjusted analysis, TNF-α and IL-6 levels were significantly associated with PTH (Table S2). The rest of the cytokines that we measured (IL-1β, IL-4, IL-8, IL-10, IL-12, IL-13, and interferon-γ) were not associated with PTH. Given the known inverse relationship between GFR and inflammation in CKD [16], we adjusted our analysis for GFR. In this adjusted analysis, TNF-α remained significantly associated with PTH, while the association between IL-6 and PTH was no longer significant (Fig. 1).

Clinical correlates of TNF-α in children with CKD

Significant differences between children from different TNF-α tertiles were found in PTH, serum phosphorus, ALP, BAP, P1NP, and height *z*-score (Fig. 2). There were no differences in age, sex, race/ethnicity, CKD etiology, GFR, 25(OH)D, and systolic and diastolic blood pressure between children from different TNF-α tertiles (Table 2). The percentages of children receiving calcitriol, nutritional Vitamin D, phosphate binders, and growth hormone were not significantly different between TNF-α tertiles.

Association between TNF-α, parameters of CKD-BMD, and growth

In multivariate analyses, PTH, ALP, serum phosphorus, and height *z*-score were significantly associated with TNF-α in all models (Table 3). BAP was significantly associated with TNF-α in models 1–3 and showed a trend toward significance ($p = 0.052$) in model 4. Similarly, P1NP was significantly associated with TNF-α in models 1 and 2 and showed a trend toward significance in models 3 ($p = 0.052$) and 4 ($p = 0.06$). Association between TNF-α and markers of CKD-MBD also remained significant after adjustment for rhGH use (Table S3). Thus, our multivariate analyses indicated that the relationship between TNF-α and CKD-BMD parameters, as well between TNF-α and height in children, was independent of age, sex, kidney function, and use of active vitamin D analogs.

Discussion

The pathophysiology of CKD-MBD and interrelated growth impairment in children with CKD remains incompletely understood. Specifically, the role of inflammation in the development of CKD-MBD and growth failure in pediatric CKD has not been elucidated. In this study, we identified a positive independent association between TNF-α and biomarkers of CKD-MBD, and an inverse association between TNF-α and height z-score in a cohort of children with CKD.

Cytokine profiling in our cohort identified TNF-α as the only cytokine associated with PTH after adjustment for GFR, among the 9 cytokines that we measured. Egli-Spichtig et al. recently conducted a similar cytokine profiling in an adult cohort and reported that TNF-α was the only cytokine associated with FGF23 [24]. IL-6, IL-1β, and IFN-γ were not associated with FGF23 in the study of Egli-Spichtig et al. and were not associated with PTH in our study. Interestingly, Glosse et al. demonstrated that TNF-α mediated the stimulatory effect of high-fat diet on FGF23 formation in mice [31]. However, the role of TNF-α in the development of CKD-MBD is likely not exclusive, and other cytokines may

Table 1 Clinical characteristics of the study cohort

Variables	Values, *n* (%) or median [IQR] (*n* = 63)
Age, years	12.3 [7.2–17.0]
Male sex	38 (60.3%)
Hispanic ethnicity	19 (30.1%)
Black race	14 (22.2%)
Glomerular etiology	16 (25.4%)
GFR, mL/min/1.73 m^2	42.0 [30.1–53.4]
CKD stage	
Stage II	15 (23.8%)
Stage III	33 (52.4%)
Stage IV	9 (14.3%)
Stage V	6 (9.5%)
Weight *z*-score	– 0.32 [– 1.25–0.82]
Height *z*-score	– 1.01 [– 2.1–0.04]
BMI *z*-score	0.62 [– 0.32–1.4]
Systolic BP percentile	57.1 [40.7–84.5]
Diastolic BP percentile	60.5 [30.4–80.2]
Phosphorus, mg/dL	4.5 [3.7–5.0]
PTH, ng/L	97 [54–295]
25(OH) vitamin D, ng/mL	27.9 [19.9–32.0]
Alkaline phosphatase, IU/L	216 [119–275]
Bone alkaline phosphatase, μg/L	63.1 [37.5–75.5]
P1NP, μg/L	538.7 [181.3–608.6]
Medication use	
Iron therapy	24 (38.1%)
ESA therapy	6 (9.5%)
Active vitamin D analogs	21 (33.3%)
Nutritional vitamin D	22 (34.9%)
Phosphate binders	7 (11.1%)
Alkalinization agents	14 (22.2%)
Growth hormone	11 (17.4%)

IQR interquartile range, *GFR* glomerular filtration rate, *BMI* body mass index, *PTH* parathyroid hormone, *P1NP* procollagen type 1 N-terminal propeptide, *ESA* erythropoiesis stimulating agents

also play a role. Indeed, McKnight et al. recently reported that IL-1β stimulated production of FGF23 at the onset of CKD in juvenile mice [32]. Wallquist et al. found that IL-12 and RANTES correlated with FGF23 in adult patients with pre-dialysis CKD [33]. Nevertheless, among several cytokines that were implicated in CKD-MBD, TNF-α has emerged as a promising target, which was consistent with our findings.

The link between inflammation and mineral metabolism has been previously reported in adult patients with CKD [22, 34]. Specifically, the association between TNF-α and serum phosphorus has been reported in adults with CKD [35]. However, this association has not been previously reported in children with CKD. In our pediatric study, we identified significant independent associations between TNF-α and several biomarkers of CKD-MBD, including serum phosphorus, PTH, total and bone-specific alkaline phosphatase, and P1NP. Vitamin D was reported to act as an inhibitor of inflammation in CKD [36–38]. However, we did not find an association between TNF-α and serum 25(OH) vitamin D in our cohort.

While the pathways linking TNF-α to CKD-MBD remain to be fully elucidated, there are several possible mechanisms by which TNF-α could be aggravating CKD-MBD. TNF-α inhibits the expression of RUNX2, a major transcription factor associated with osteoblast differentiation [39]. At the same time, TNF-α is a potent stimulator of osteoclastogenesis [40]. Furthermore, inflammation appears to stimulate production of FGF23 in CKD, both directly through upregulating FGF23 production and cleavage by osteocytes, as well as indirectly through activation of hepcidin and induction of hypoferremia, among other potential mechanisms [41]. Further studies are needed to delineate the mechanisms

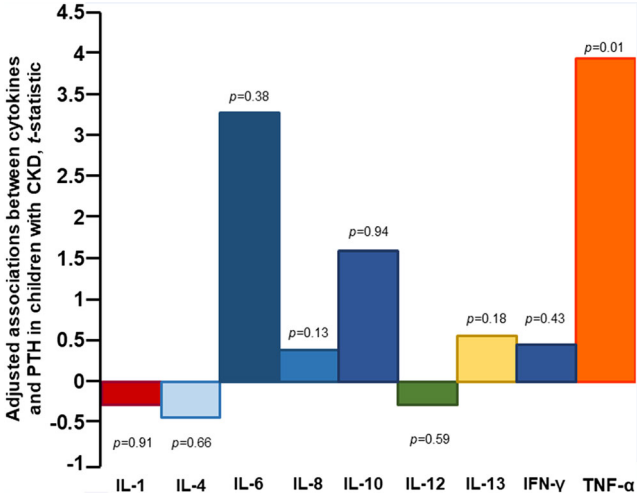

Fig. 1 Cytokine profiling and PTH in children with CKD. This figure depicts the associations between cytokines and parathyroid hormone (PTH) in children with chronic kidney disease (CKD). Each bar represents a separate regression analysis of the PTH on respective cytokine, adjusted for glomerular filtration rate; *t*-statistic values corresponding to the significance levels (*P*) for the overall models are graphed. Negative values correspond to inverse correlations. $n = 63$. IFN, interferon; IL, interleukin; TNFα, tumor necrosis factor alpha

underlying the effects of TNF-α in CKD-MBD, particularly elucidating the role of soluble receptors for TNF-α.

The possible role of inflammation in growth impairment of children with CKD has been acknowledged in the literature [42]. The growth-inhibiting effect of inflammation has been traditionally explained by a suppressive action of inflammation on the nutritional status. TNF-α exhibits well recognized anorexigenic effects, which gave TNF-α its synonymous name, cachectin. Indeed, anorexia was observed following acute administration of TNF-α to mice [43]. Administration of recombinant TNF-α increased energy expenditure in patients [44]. In a meta-analysis, serum levels of TNF-α were elevated in patients with anorexia nervosa compared to controls [45]. In patients with CKD 5, TNF-α and its genetic variants were the major contributors to the development of malnutrition inflammation syndrome [46]. Inflammation may affect growth also by disrupting the growth hormone (GH), insulin-like growth factor 1 (IGF1) signaling. In patients with chronic liver disease, TNF-α appeared to be a major factor responsible for acquired GH resistance [47]. Children with sickle cell disease who had higher TNF-α suffered from more severe growth impairment [48]. Despite this evidence, the direct association between inflammation and growth delay in children with CKD has not been established to date. In our study, we found an inverse association between TNF-α and height *z*-score in children with CKD, which was independent of GFR. These data provide previously missing direct evidence for the role of inflammation in growth impairment of children with CKD. The suppressive effect of TNF-α on growth could be mediated by its effects on bone health, nutritional status, and GH-IGF1 signaling.

Whereas experience with anti-cytokine therapies in children with CKD has been limited [11], they have been widely and successfully used in other inflammatory conditions. Several TNF-α inhibitors are FDA-approved for various indications, including monoclonal antibodies such as infliximab

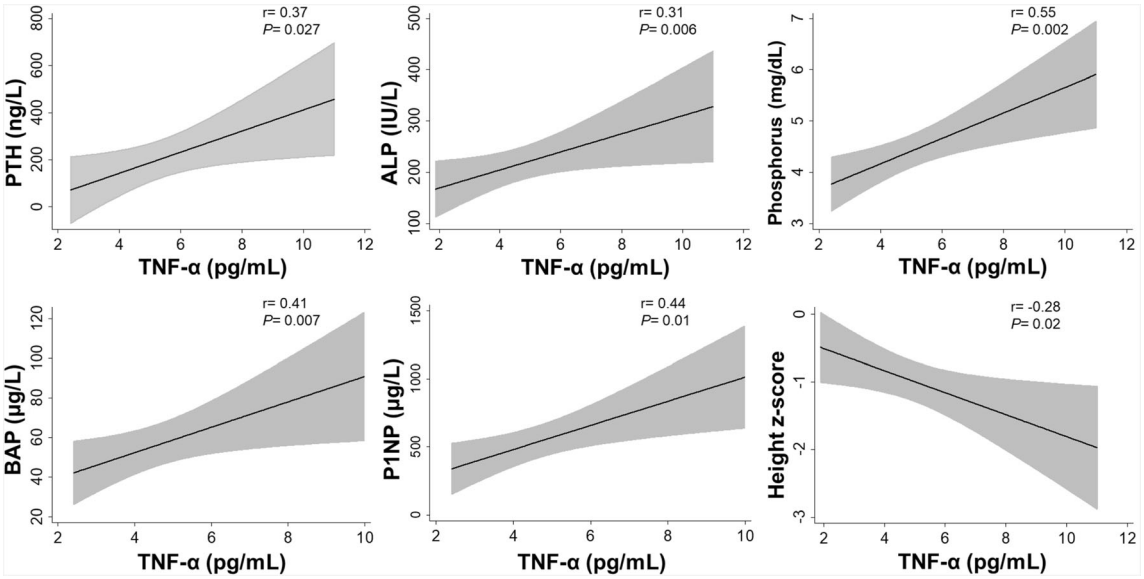

Fig. 2 Associations between TNF-α and biomarkers of chronic kidney disease-mineral and bone disorder and growth in children with chronic kidney disease: **a** parathyroid hormone (PTH), **b** alkaline phosphatase (ALP), **c** phosphorus, **d** bone alkaline phosphatase (BAP), and **e** procollagen type 1 N-terminal propeptide (P1NP) each positively correlate with TNF-α. **f** Height z-score inversely correlates with TNF-α. $n = 63$. Gray area surrounding black regression lines represents 95% confidence interval

Table 2 Characteristics of bone health and growth stratified by TNF-α tertiles

Cohort characteristics	TNF-α tertiles (pg/mL)			*P*
	I [1.9 to 3.5] (*n* = 21)	II [3.6 to 4.9] (*n* = 21)	III [5.0 to 11.0] (*n* = 21)	
GFR, mL/min/1.73 m^2	46.9 [38.0 to 50.2]	46.2 [32.5 to 63.5]	35.8 [26.2 to 48.9]	0.467
Phosphorus, mg/dL	3.9 (3.6 to 4.6)	4.3 (3.7 to 5.2)	4.8 (4.4 to 5.2)	0.02
PTH, ng/L	57 (25 to 127)	91 (46 to 98)	223 (98 to 454)	0.003
25(OH) vitamin D, ng/mL	23.4 (17.5 to 31.8)	29.70 (23.6 to 35.5)	28.60 (21.8 to 35.0)	0.35
Alkaline phosphatase, IU/L	119 (82 to 218)	218 (181 to 266)	270 (170 to 365)	0.04
Bone alkaline phosphatase, μg/L	15.7 (11.8 to 28.7)	63.7 (49.2 to 71.0)	77.0 (64.8 to 96.5)	0.009
P1NP, μg/L	152.7 (98.7 to 195.3)	452.1 (197.7 to 566.9)	691.0 (556.6 to 935.9)	< 0.001
Medication use:				
Active vitamin D analogs	4 (19%)	7 (33.3%)	10 (47.6%)	0.12
Nutritional vitamin D	7 (41.2%)	10 (58%)	5 (47.1%)	0.57
Phosphate binders	0 (0%)	2 (8.7%)	5 (21.7%)	0.12
Growth hormone	4 (23.5%)	4 (23.5%)	3 (17.6%)	0.59
Height *z*-score	0.02 [– 0.32 to 1.28]	– 1.19 [– 1.68 to – 1.01]	– 2.33 [– 2.41 to – 2.10]	0.04

Continuous variables presented as median (interquartile range)

TNF-α tumor necrosis factor alpha, *GFR* glomerular filtration rate, *PTH* parathyroid hormone, *P1NP* procollagen type 1 N-terminal propeptide

and adalimumab, and receptor fusion protein (etanercept). In several chronic inflammatory disorders of childhood, studies have established protective effects of TNF-α inhibitors against bone disease and growth impairment. In children with inflammatory bowel disease, anti TNF-α therapy improved trabecular bone mineral density and cortical structure [49], as well as statural growth, which was not solely due to a steroid sparing effect [50]. TNF-α inhibition ameliorated bone loss [51] and restored impaired growth velocity in children with juvenile idiopathic arthritis [52].

The strengths of this study included unbiased cytokine profiling and comprehensive assessment of both traditional and novel biomarkers of CKD-MBD in a well-defined cohort of children with CKD. Our study had limitations, including single center cohort and relatively small sample size, as well as not assessing FGF23. We had only 11 children who were receiving rhGH in the cohort. Establishing causality was beyond the scope of our study. The relationship between inflammation and CKD-MBD may be bi-directional. Indeed, FGF23 directly stimulates production of pro-inflammatory cytokines by the liver in CKD [53]. Dietary phosphate loading increased serum TNF-α in uremic rats [54]. After parathyroidectomy, bone expression of TNF-α significantly decreases in hemodialysis patients [55]. Multicenter and prospective clinical studies, as well as additional basic science work, would be warranted to further elucidate the role of TNF-α in pediatric CKD.

Table 3 Multivariate analysis of the relationship between serum TNF-α, parameters of CKD-MBD, and height

Parameters	Model 1		Model 2		Model 3		Model 4	
	Unadjusted		Adjust: model 1+ age + sex		Adjust: model 2 + GFR		Adjust: model 2 + active vitamin D use	
	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*	β (95% CI)	*P*
PTH	45.0 (5.4, 84.6)	0.02	61.6 (20.0, 103.2)	0.005	53.2 (18.9, 97.8)	0.03	57.3 (16.8, 97.9)	0.007
ALP	39.6 (26.1, 51.2)	< 0.001	35.8 (16.9, 46.8)	0.02	30.6 (3.5, 42.2)	0.03	28.6 (3.8, 43.2)	0.03
Phosphorus	0.21 (0.04, 0.37)	0.013	0.22 (0.04, 0.39)	0.016	0.27 (0.11, 0.44)	0.003	0.25 (0.05, 0.44)	0.01
BAP	11.8 (5.1, 18.5)	0.001	7.8 (4.8, 15.7)	0.04	7.8 (4.8, 15.7)	0.04	7.9 (– 0.09, 15.8)	0.052
P1NP	121.3 (54.1, 188.4)	0.001	85.4 (– 3.1, 145.2)	0.04	81.2 (– 3.1, 143.4)	0.052	79.2 (– 9.0, 143.4)	0.06
Height *z*-score	– 0.46 (– 0.62, – 0.31)	< 0.001	– 0.40 (– 0.59, – 0.21)	< 0.001	– 0.44 (– 0.69, – 0.18)	0.002	– 0.44 (0.05, 0.44)	0.002

CI confidence Interval, *PTH* parathyroid hormone, *ALP* alkaline phosphatase, *BAP* bone alkaline phosphatase, *P1NP* procollagen type 1 N-terminal propeptide.

In conclusion, this study demonstrates an independent positive association between circulating TNF-α and several biomarkers of CKD-MBD in children with CKD. Furthermore, we found an inverse independent association between TNF-α and height *z*-score in our cohort. These data call for attention to the role of TNF-α in bone health and growth in children with CKD and warrant studies that would examine the potential of TNF-α as a therapeutic target in CKD.

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References

1. Saran R, Robinson B, Abbott KC, Bragg-Gresham J, Chen X, Gipson D, Gu H, Hirth RA, Hutton D, Jin Y, Kapke A, Kurtz V, Li Y, McCullough K, Modi Z, Morgenstern H, Mukhopadhyay P, Pearson J, Pisoni R, Repeck K, Schaubel DE, Shamraj R, Steffick D, Turf M, Woodside KJ, Xiang J, Yin M, Zhang X, Shahinian V (2020) US renal data system 2019 annual data report: epidemiology of kidney disease in the United States. Am J Kidney Dis 75(1 Suppl 1):A6–A7
2. Borzych D, Rees L, Ha IS, Chua A, Valles PG, Lipka M, Zambrano P, Ahlenstiel T, Bakkaloglu SA, Spizzirri AP, Lopez L, Ozaltin F, Printza N, Hari P, Klaus G, Bak M, Vogel A, Ariceta G, Yap HK, Warady BA, Schaefer F, International Pediatric PD Network (IPPN) (2010) The bone and mineral disorder of children undergoing chronic peritoneal dialysis. Kidney Int 78:1295–1304
3. Wesseling-Perry K, Pereira RC, Tseng CH, Elashoff R, Zaritsky JJ, Yadin O, Sahney S, Gales B, Jüppner H, Salusky IB (2012) Early skeletal and biochemical alterations in pediatric chronic kidney disease. Clin J Am Soc Nephrol 7:146–152
4. Portale AA, Wolf M, Jüppner H, Messinger S, Kumar J, Wesseling-Perry K, Schwartz GJ, Furth SL, Warady BA, Salusky IB (2014) Disordered FGF23 and mineral metabolism in children with CKD. Clin J Am Soc Nephrol 9:344–353
5. Denburg MR (2016) Fracture burden and risk factors in childhood CKD: results from the CKiD cohort study. J Am Soc Nephrol 27:543–550
6. Faul C, Amaral AP, Oskouei B, Hu MC, Sloan A, Isakova T, Gutiérrez OM, Aguillon-Prada R, Lincoln J, Hare JM, Mundel P, Morales A, Scialla J, Fischer M, Soliman EZ, Chen J, Go AS, Rosas SE, Nessel L, Townsend RR, Feldman HI, St John Sutton M, Ojo A, Gadegbeku C, Di Marco GS, Reuter S, Kentrup D, Tiemann K, Brand M, Hill JA, Moe OW, Kuro-O M, Kusek JW, Keane MG, Wolf M (2011) FGF23 induces left ventricular hypertrophy. J Clin Invest 121:4393–4408
7. Staude H, Jeske S, Schmitz K, Warncke G, Fischer DC (2013) Cardiovascular risk and mineral bone disorder in patients with chronic kidney disease. Kidney Blood Press Res 37:68–83
8. Wesseling-Perry K, Salusky IB (2013) Chronic kidney disease: mineral and bone disorder in children. Semin Nephrol 33:169–179
9. Waller SC, Ridout D, Cantor T, Rees L (2005) Parathyroid hormone and growth in children with chronic renal failure. Kidney Int 67:2338–2345
10. Seikaly MG, Salhab N, Gipson D, Yiu V, Stablein D (2006) Stature in children with chronic kidney disease: analysis of NAPRTCS database. Pediatr Nephrol 21:793–799
11. Akchurin OM, Kaskel F (2015) Update on inflammation in chronic kidney disease. Blood Purif 39:84–92
12. Gupta J, Mitra N, Kanetsky PA, Devaney J, Wing MR, Reilly M, Shah VO, Balakrishnan VS, Guzman NJ, Girndt M, Periera BG, Feldman HI, Kusek JW, Joffe MM, Raj DS, CRIC Study Investigators (2012) Association between albuminuria, kidney function, and inflammatory biomarker profile in CKD in CRIC. Clin J Am Soc Nephrol 7:1938–1946
13. Akchurin O, Patino E, Dalal V, Meza K, Bhatia D, Brovender S, Zhu YS, Cunningham-Rundles S, Perelstein E, Kumar J, Rivella S, Choi ME (2018) Interleukin-6 contributes to the development of anemia in juvenile CKD. Kidney Int Rep 4:470–483
14. Fardellone P, Séjourné A, Paccou J, Goëb V (2014) Bone remodelling markers in rheumatoid arthritis. Mediat Inflamm 2014:484280
15. Sgambato D, Gimigliano F, De Musis C, Moretti A, Toro G, Ferrante E, Miranda A, De Mauro D, Romano L, Iolascon G, Romano M (2019) Bone alterations in inflammatory bowel diseases. World J Clin Cases 7:1908–1925
16. Shaw AT, Gravallese EM (2016) Mediators of inflammation and bone remodeling in rheumatic disease. Semin Cell Dev Biol 49:2–10
17. Tambralli A, Beukelman T, Weiser P, Atkinson TP, Cron RQ, Stoll ML (2013) High doses of infliximab in the management of juvenile idiopathic arthritis. J Rheumatol 40:1749–1755
18. Rinawi F, Assa A, Almagor T, Ziv-Baran T, Shamir R (2019) Prevalence and predictors of growth impairment and short stature in pediatric-onset inflammatory bowel disease. Digestion. https://doi.org/10.1159/000501924
19. Guzman J, Kerr T, Ward LM, Ma J, Oen K, Rosenberg AM, Feldman BM, Boire G, Houghton K, Dancey P, Scuccimarri R, Bruns A, Huber AM, Watanabe Duffy K, Shiff NJ, Berard RA, Levy DM, Stringer E, Morishita K, Johnson N, Cabral DA, Larché M, Petty RE, Laxer RM, Silverman E, Miettunen P, Chetaille AL, Haddad E, Spiegel L, Turvey SE, Schmeling H, Lang B, Ellsworth J, Ramsey SE, Roth J, Campillo S, Benseler S, Chédeville G, Schneider R, Tse SML, Bolaria R, Gross K, Feldman D, Cameron B, Jurencak R, Dorval J, LeBlanc C, St Cyr C, Gibbon M, Yeung RSM, Duffy CM, Tucker LB (2017) Growth and weight gain in children with juvenile idiopathic arthritis: results from the ReACCh-Out cohort. Pediatr Rheumatol Online J 15:68
20. Maldonado-Pérez MB, Castro-Laria L, Caunedo-Álvarez A, Montoya-García MJ, Giner-García M, Argüelles-Arias F, Romero-Gómez M, Vázquez-Gámez MÁ (2019) Does the antitumor necrosis factor-alpha therapy decrease the vertebral fractures occurrence in inflammatory bowel disease? J Clin Densitom 22:195–202
21. Horneff G, Burgos-Vargas R, Constantin T, Foeldvari I, Vojinovic J, Chasnyk VG, Dehoorne J, Panaviene V, Susic G, Stanevica V, Kobusinska K, Zuber Z, Mouy R, Rumba-Rozenfelde I, Breda L, Dolezalova P, Job-Deslandre C, Wulffraat N, Alvarez D, Zang C, Wajdula J, Woodworth D, Vlahos B, Martini A, Ruperto N, Paediatric Rheumatology International Trials Organisation (PRINTO) (2014) Efficacy and safety of open-label etanercept on extended oligoarticular juvenile idiopathic arthritis, enthesitis-

related arthritis and psoriatic arthritis: part 1 (week 12) of the CLIPPER study. Ann Rheum Dis 73:1114–1122
22. Navarro-González JF, Mora-Fernández C, Muros M, Herrera H, García J (2009) Mineral metabolism and inflammation in chronic kidney disease patients: a cross-sectional study. Clin J Am Soc Nephrol 4:1646–1654
23. Munoz Mendoza J, Isakova T, Ricardo AC, Xie H, Navaneethan SD, Anderson AH, Bazzano LA, Xie D, Kretzler M, Nessel L, Hamm LL, Negrea L, Leonard MB, Raj D, Wolf M, Chronic renal insufficiency cohort (2012) Fibroblast growth factor 23 and Inflammation in CKD. Clin J Am Soc Nephrol 7:1155–1162
24. Egli-Spichtig D, Imenez Silva PH, Glaudemans B, Gehring N, Bettoni C, Zhang MYH, Pastor-Arroyo EM, Schönenberger D, Rajski M, Hoogewijs D, Knauf F, Misselwitz B, Frey-Wagner I, Rogler G, Ackermann D, Ponte B, Pruijm M, Leichtle A, Fiedler GM, Bochud M, Ballotta V, Hofmann S, Perwad F, Föller M, Lang F, Wenger RH, Frew I, Wagner CA (2019) Tumor necrosis factor stimulates fibroblast growth factor 23 levels in chronic kidney disease and non-renal inflammation. Kidney Int 96:890–905
25. Viaene L, Behets GJ, Heye S, Claes K, Monbaliu D, Pirenne J, D'Haese PC, Evenepoel P (2016) Inflammation and the bone-vascular axis in end-stage renal disease. Osteoporos Int 27:489–497
26. Sang C, Zhang Y, Chen F, Huang P, Qi J, Wang P, Zhou Q, Kang H, Cao X, Guo L (2016) Tumor necrosis factor alpha suppresses osteogenic differentiation of MSCs by inhibiting semaphorin 3B via Wnt/β-catenin signaling in estrogen-deficiency induced osteoporosis. Bone 84:78–87
27. Andrassy KM (2013) Comments on 'KDIGO 2012 Clinical Practice Guideline for the evaluation and management of chronic kidney disease'. Kidney Int 84:622–623
28. Mian AN, Schwartz GJ (2017) Measurement and estimation of glomerular filtration rate in children. Adv Chronic Kidney Dis 24: 348–356
29. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Strawn LM, Flegal KM, Mei Z, Wei R, Curtin LR, Roche AF, Johnson CL (2002) 2000 CDC growth charts for the United States: methods and development. Vital Health Stat 11:1–190
30. Hulley S, Cummings SR, Browner WS, Grady DG, Newman TB (2013) Designing clinical research, vol 4th. LWW, Philadelphia
31. Glosse P, Fajol A, Hirche F, Feger M, Voelkl J, Lang F, Stangl GI, Föller M (2018) A high-fat diet stimulates fibroblast growth factor 23 formation in mice through TNFα upregulation. Nutr Diabetes 8: 36
32. McKnight Q, Jenkins S, Li X, Nelson T, Marlier A, Cantley LG, Finberg KE, Fretz JA (2020) IL-1β drives production of FGF-23 at the onset of chronic kidney disease in mice. J Bone Miner Res 35: 1352–1362
33. Wallquist C, Mansouri L, Norrbäck M, Hylander B, Jacobson SH, Larsson TE, Lundahl J (2018) Associations of fibroblast growth factor 23 with markers of inflammation and leukocyte transmigration in chronic kidney disease. Nephron 138:287–295
34. Lee C-T, Tsai YC, Ng HY, Su Y, Lee WC, Lee LC, Chiou TT, Liao SC, Hsu KT (2009) Association between C-reactive protein and biomarkers of bone and mineral metabolism in chronic hemodialysis patients: a cross-sectional study. J Ren Nutr 19:220–227
35. Vázquez-Huerta DI, Alvarez-Rodríguez BA, Topete-Reyes JF, Muñoz-Valle JF, Parra-Michel R, Fuentes-Ramírez F, Salazar-López MA, Valle Y, Reyes-Castillo Z, Cruz-González A, Brennan-Bourdon LM, Torres-Carrillo N (2014) Tumor necrosis factor alpha-238 G/A and-308 G/A polymorphisms and soluble TNF-α levels in chronic kidney disease: correlation with clinical variables. Int J Clin Exp Med 7:2111–2119
36. Sanchez-Niño MD, Bozic M, Córdoba-Lanús E, Valcheva P, Gracia O, Ibarz M, Fernandez E, Navarro-Gonzalez JF, Ortiz A, Valdivielso JM (2012) Beyond proteinuria: VDR activation reduces renal inflammation in experimental diabetic nephropathy. Am J Physiol Ren Physiol 302:F647–F657
37. Paul K, Franke S, Nadal J, Schmid M, Yilmaz A, Kretzschmar D, Bärthlein B, Titze S, Koettgen A, Wolf G, Busch M, GCKD study group (2016) Inflammation, vitamin D and dendritic cell precursors in chronic kidney disease. Clin Exp Immunol 186:86–95
38. Querfeld U (2013) Vitamin D and inflammation. Pediatr Nephrol 28:605–610
39. Ding J, Ghali O, Lencel P, Broux O, Chauveau C, Devedjian JC, Hardouin P, Magne D (2009) TNF-α and IL-1β inhibit RUNX2 and collagen expression but increase alkaline phosphatase activity and mineralization in human mesenchymal stem cells. Life Sci 84: 499–504
40. Lam J, Takeshita S, Barker JE, Kanagawa O, Ross FP, Teitelbaum SL (2000) TNF-α induces osteoclastogenesis by direct stimulation of macrophages exposed to permissive levels of RANK ligand. J Clin Invest 106:1481–1488
41. David V, Martin A, Isakova T, Spaulding C, Qi L, Ramirez V, Zumbrennen-Bullough KB, Sun CC, Lin HY, Babitt JL, Wolf M (2016) Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. Kidney Int 89:135–146
42. Tu J, Cheung WW, Mak RH (2016) Inflammation and nutrition in children with chronic kidney disease. World J Nephrol 5:274–282
43. Wu W, Zhang H (2014) Role of tumor necrosis factor-α and interleukin-1β in anorexia induction following oral exposure to the trichothecene deoxynivalenol (vomitoxin) in the mouse. J Toxicol Sci 39:875–886
44. Starnes HF Jr, Warren RS, Jeevanandam M, Gabrilove JL, Larchian W, Oettgen HF, Brennan MF (1988) Tumor necrosis factor and the acute metabolic response to tissue injury in man. J Clin Invest 82: 1321–1325
45. Solmi M, Veronese N, Favaro A, Santonastaso P, Manzato E, Sergi G, Correll CU (2015) Inflammatory cytokines and anorexia nervosa: a meta-analysis of cross-sectional and longitudinal studies. Psychoneuroendocrinology 51:237–252
46. Sharma R, Agrawal S, Saxena A, Sharma RK (2013) Association of IL-6, IL-10, and TNF-α gene polymorphism with malnutrition inflammation syndrome and survival among end stage renal disease patients. J Interf Cytokine Res 33:384–391
47. Picardi A, Gentilucci UV, Zardi EM, Caccavo D, Petitti T, Manfrini S, Pozzilli P, Afeltra A (2003) TNF-α and growth hormone resistance in patients with chronic liver disease. J Interf Cytokine Res 23:229–235
48. Kuvibidila S, Gardner R, Ode D, Yu L, Lane G, Warrier RP (1997) Tumor necrosis factor alpha in children with sickle cell disease in stable condition. J Natl Med Assoc 89:609–615
49. Griffin LM, Thayu M, Baldassano RN, DeBoer MD, Zemel BS, Denburg MR, Denson LA, Shults J, Herskovitz R, Long J, Leonard MB (2015) Improvements in bone density and structure during anti-TNF-alpha therapy in pediatric Crohn's disease. J Clin Endocrinol Metab 100:2630–2639
50. Malik S, Ahmed SF, Wilson ML, Shah N, Loganathan S, Naik S, Bourke B, Thomas A, Akobeng AK, Fagbemi A, Wilson DC, Russell RK (2012) The effects of anti-TNF-alpha treatment with adalimumab on growth in children with Crohn's disease (CD). J Crohns Colitis 6:337–344
51. Simonini G, Giani T, Stagi S, de Martino M, Falcini F (2005) Bone status over 1 yr of etanercept treatment in juvenile idiopathic arthritis. Rheumatology (Oxford) 44:777–780
52. Tynjälä P, Lahdenne P, Vähäsalo P, Kautiainen H, Honkanen V (2006) Impact of anti-TNF treatment on growth in severe juvenile idiopathic arthritis. Ann Rheum Dis 65:1044–1049
53. Singh S, Grabner A, Yanucil C, Schramm K, Czaya B, Krick S, Czaja MJ, Bartz R, Abraham R, Di Marco GS, Brand M, Wolf M, Faul C (2016) Fibroblast growth factor 23 directly targets

hepatocytes to promote inflammation in chronic kidney disease. Kidney Int 90:985–996

54. Yamada S, Tokumoto M, Tatsumoto N, Taniguchi M, Noguchi H, Nakano T, Masutani K, Ooboshi H, Tsuruya K, Kitazono T (2014) Phosphate overload directly induces systemic inflammation and malnutrition as well as vascular calcification in uremia. Am J Physiol Ren Physiol 306:F1418–F1428

55. Santos FRL, Moysés RMA, Montenegro FLM, Jorgetti V, Noronha IL (2003) IL-1β, TNF-α, TGF-β, and bFGF expression in bone biopsies before and after parathyroidectomy. Kidney Int 63:899–907