Association between indoxyl sulfate and bone histomorphometry in pre-dialysis chronic kidney disease patients

Associação entre indoxil sulfato e histomorfometria óssea em pacientes renais crônicos pré-diálise

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Abstract

Introduction: Experimental studies have suggested that indoxyl sulfate (IS), a protein-bound uremic toxin, may be involved in the development of renal osteodystrophy. **Objective:** evaluate the association between IS levels and biochemical parameters related to mineral metabolism and bone histomorphometry in a cohort of pre-dialysis chronic kidney disease (CKD) patients. **Methods:** This is a post-hoc analysis of an observational study evaluating the association between coronary calcification and bone biopsy findings in 49 patients (age: 52 ± 10 years; 67% male; estimated glomerular filtration rate: 36 ± 17 ml/min). Serum levels of IS were measured. **Results:** Patients at CKD stages 2 and 3 presented remarkably low bone formation rate. Patients at CKD stages 4 and 5 presented significantly higher osteoid volume, osteoblast and osteoclast surface, bone fibrosis volume and bone formation rate and a lower mineralization lag time than CKD stage 2 and 3 patients. We observed a positive association between IS levels on one hand and the bone formation rate, osteoid volume, osteoblast surface and bone fibrosis volume on the other. Multivariate regression models confirmed that the associations between IS levels and osteoblast surface and bone fibrosis volume were both independent of demographic and biochemical characteristics of the study population. A similar trend was observed for the bone formation rate. **Conclusions:** Our findings demonstrated that IS is positively associated with bone formation rate in pre-dialysis CKD patients.

Keywords: renal insufficiency, chronic; indoxyl sulfate; renal osteodystrophy; uremia.

Resumo

Introdução: Estudos experimentais indicam que o indoxil sulfato (IS), uma toxina urêmica ligada à proteína, pode estar envolvido no desenvolvimento da osteodistrofia renal. **Objetivo:** Avaliar a associação entre os níveis séricos de IS e parâmetros bioquímicos do metabolismo mineral e da histomorfometria óssea em uma coorte de pacientes com doença renal crônica (DRC) pré-diálise. **Métodos:** Análise *post-hoc* de um estudo que avaliou a associação entre calcificação coronariana e histomorfometria óssea em 49 pacientes (idade: 52 ± 10 anos; 67% sexo masculino; taxa de filtração glomerular estimada: 36 ± 17 ml/min). Os níveis séricos de IS foram dosados. **Resultados:** Pacientes com DRC estágio 2 e 3 apresentaram uma taxa de formação óssea baixa. Pacientes com DRC estágio 4 e 5 apresentaram volume osteoide, superfícies osteoblástica e osteoclástica, volume de fibrose e taxa de formação óssea significativamente maiores e intervalo de mineralização significativamente menor que os pacientes com DRC estágio 2 e 3. Os níveis séricos de IS associaram-se positivamente com a taxa de formação óssea, volume osteoide, superfície osteoblástica e volume de fibrose. A análise de regressão multivariada identificou que o IS é um fator independente determinante da superfície osteoblástica e fibrose. Uma tendência similar foi observada para a taxa de formação óssea. **Conclusão:** Nosso estudo sugere que, na DRC pré-dialítica, o IS correlaciona-se positivamente com a formação óssea.

Palavras-chave: indoxil sulfato; insuficiência renal crônica; osteodistrofia renal; uremia.

Introduction

Disturbances of bone and mineral metabolism are common in chronic kidney disease (CKD) patients and are associated with increased morbidity and reduced quality of life.[1,2] The various pathological bone alterations found in CKD patients are collectively referred to as renal osteodystrophy (ROD) and include alterations in bone histology, which can be quantified by the histomorphometric assessment of bone biopsies.

It is known that ROD begins very early in the course of CKD.[3,4] Many factors have been incriminated in the pathophysiology of this condition, including calcium, phosphate, parathyroid hormone (PTH), vitamin D and its analogues, fibroblast growth factor-23 (FGF-23) and, more recently, sclerostin.[5] Several other important, quantifiable solutes that have negative biological effects and are retained in the body as the uremic condition progresses - the so-called uremic toxins - have been implicated as well.

Indoxyl sulfate (IS) is a protein-bound uremic toxin derived from the metabolism of dietary tryptophan. Briefly, tryptophan is metabolized into indole by intestinal bacteria and, after intestinal absorption, is further converted to IS in the liver. It is excreted by the kidneys via proximal tubular secretion. Consequently, IS accumulates in the blood of patients with impaired renal function. Moreover, IS's high binding affinity for albumin means that it cannot be efficiently removed by conventional hemodialysis.[6] The role of IS as a uremic toxin was first revealed by its accelerating effects on CKD progression,[7] possibly via a reduction in proximal tubular cell viability due to increased oxidative stress.[8] Iwazaki *et al.*[9] subsequently used a rat model of kidney failure associated with low bone turnover to show that IS accumulation was related to lower bone formation rate and the down-regulation of osteoblast-related genes. This condition was improved by treatment with the oral adsorbent AST-120 (probably by reducing IS levels). The same group subsequently showed that IS reduced PTH-induced cAMP production, PTH receptor (PTH1R) gene expression and cell viability in primary osteoblast cultures.[10] More recently, Mozar *et al.*[11] have demonstrated that IS inhibits osteoclast differentiation and function. In the clinical setting, Goto *et al.*[12] have reported a negative association between IS and serum markers of bone turnover, independent of PTH levels, in a cohort of 47 hemodialysis patients. These observations prompted the hypothesis whereby accumulation of IS is involved in the development of renal osteodystrophy. To date, no study assessed by bone histomorphometry has been performed to evaluate this hypothesis.

The present study sought to evaluate the association between circulating indoxyl sulfate (IS), a protein-bound uremic toxin, and biochemical parameters related to mineral metabolism [such as calcium, phosphate, bone-specific alkaline phosphatase, intact-PTH, FGF-23, 25(OH) vitamin D_3 (25D) and 1,25 $(OH)_2$ vitamin D_3 (1,25 D)] and histomorphometric parameters in a cohort of prevalent, treatment-naïve pre-dialysis CKD patients.

Material and methods

Subjects and study design

This is a post-hoc analysis of a cross-sectional study that evaluated the association between coronary artery calcification and bone histomorphometric parameters in a cohort of prevalent, pre-dialysis CKD patients from an outpatient nephrology clinic in São Paulo, Brazil.[13] Patients were 18 years old or over, with 24-hour creatinine clearance rates of between 15 and 90 ml/min/m^2. All had been monitored by a nephrologist for at least 3 months and had not received any phosphate binders, vitamin D analogues or corticosteroids. Included patients underwent clinical and physical examinations. There was no evidence of inflammatory, neoplastic or infectious disease in any subject. Weight and height were used to calculate the body mass index (BMI). For any given patient, laboratory tests and the bone biopsy were performed within 30 days of patient selection.

All patients gave their informed, written consent. The study protocol was reviewed and approved by the local institutional review board and was performed in accordance with the ethical principles of the Declaration of Helsinki.

Laboratory tests

Fasting blood samples were assayed for creatinine, ionized calcium, phosphorus, alkaline phosphatase (reference ranges: < 270 U/L for men < and 240 U/L for women), bone-specific alkaline phosphatase (enzyme immunoassay from Metra Biosystems Inc, Mountain View, CA, USA; reference range: 11.6 to 42.7 U/L for men and 15 to 41.3 U/L for women), intact PTH (iPTH; Immulite Assay, DPC, Los Angeles, CA, USA; reference range: 10 to 65

pg/ml), FGF-23 (ELISA from Kainos Laboratories, Tokyo, Japan; reference range: 28.9 ± 1.1 pg/ml), 1,25 D (radioimmunoassay, Gamma counter, Perkin Elmer, Brazil, reference range: 15.9 to 55.6 pg/ml) and 25D (radioimmunoassay, DiaSorin®, Stillwater, MN, USA; reference range: 18 to 62 ng/dL). For determination of serum total IS levels, samples were deproteinized by heat denaturation and analyzed by reverse-phase, high-performance liquid chromatography. Concentrations were then determined by fluorescence detection (excitation at 280 nm and emission at 340 nm for IS).[14] The same method was used for free IS determinations, except that serum samples were ultrafiltered through a Centrifree (Millipore), prior to deproteinization. The reference values for total and free IS in our healthy control subjects (n = 20) were 0.090 ± 0.034 mg/dL and 0.016 ± 0.004 mg/dL, respectively.

In order to estimate the true GFR as accurately as possible from serum creatinine levels, we applied the recently published "CKD-EPI" equation.[6] For descriptive purposes, patients were then classified into CKD stages according to the National Kidney Foundation's K/DOQI guidelines.[15] Once there were low numbers of patients in CKD stages 2 and 5, the study population was pooled into two groups (CKD stages 2-3 and stages 4-5) for descriptive and analytical purposes.

Bone biopsy

Bone samples were taken from the iliac crest after double tetracycline labeling. Bone fragments underwent standard histological processing.[8] Semiautomatic bone histomorphometric analysis was performed using Osteomeasure software (Osteometrics Inc, Atlanta, GA), as previously described.[13] The analyzed histomorphometric parameters were those proposed by the American Society of Bone and Mineral Research Histomorphometry Nomenclature Committee.[16] Reference ranges used for static parameters were obtained from local controls,[17] whereas dynamic parameters followed those described elsewhere.[18]

Selected histomorphometric parameters for bone turnover [bone formation rate/bone surface (BFR/BS)], mineralization [mineralization lag time (Mlt)] and volume [trabecular bone volume (BV/TV)], as well as the osteoblast surface/bone surface (Ob.S/BS), osteoid volume/bone volume (OV/BV), osteoclast surface/bone surface (Oc.S/BS), eroded surface/bone surface (ES/BS) and fibrosis volume (Fb.V) were described according to the published guidelines on ROD classification.[1]

Statistical analysis

Data are expressed as mean ± SD, median and range or frequency. For parameters presenting a non-Gaussian distribution, values were log-normalized prior to use in tests that require normally distributed variables. Intergroup comparisons were performed using a χ^2 test for categorical variables and the Student's *t*-test or the Mann-Whitney test for continuous variables. Univariate linear regression models were used to check the associations between serum total IS levels and markers of mineral metabolism, as well as bone histomorphometric parameters. Variables selected in univariate analyses were fed into multivariate linear regression models to verify the independence of identified associations. A p value ≤ 0.05 was considered to be statistically significant. All statistical analyses were performed using PASW® statistics software, version 18.0.

The authors had full access to the data and take full responsibility for their integrity. All authors have read and agreed to the manuscript as written.

Results

The patients were preponderantly male (67%) and white race (49%), with 40% presenting *diabetes mellitus* as a comorbid condition. The mean ± SD age was 52 ± 10 years and the median time since CKD diagnosis was 30 months (range: 15 to 83 months). Hypertensive nephrosclerosis was the most frequent etiology for CKD (39%), followed by diabetic nephropathy (31%). The mean estimated GFR in the study cohort was 36 ± 17 ml/min (10% at CKD stage 2.49% at CKD stage 3, 35% at CKD stage 4 and 6% at CKD stage 5, according to the National Kidney Foundation's K/DOQI guidelines).[19] Mean albumin and hemoglobin values (4.3 ± 0.4 g/dL and 13 ± 2 g/dL, respectively) were within the normal reference ranges. It is noteworthy that most of the patients (60%) had sufficient 25D levels (i.e. 25D ≥ 30 ng/ml) and only 10% of the study patients presented vitamin D deficiency (defined as 25D ≤ 15 ng/ml).[20]

Table 1 shows information on biochemical and hormonal characteristics, by CKD stage. Serum levels of phosphate, iPTH, FGF-23, bone-specific alkaline phosphatase, as well as total and free IS

were significantly elevated in patients at CKD stages 4 and 5, in comparison with CKD stage 2 and 3 patients. Additionally, serum levels of bicarbonate, 25D and 1,25 D were lower in CKD stage 4 and 5 patients than in CKD stage 2 and 3 patients. As expected (Figure 1), there was a significant, negative association between serum total IS and the eGFR. Interestingly, total and free IS serum levels were already elevated in patients at CKD stages 2 and 3, when compared with 20 healthy controls (mean ± SD total IS of 0.165 ± 0.079 *vs.* 0.090 ± 0.034 mg/dL, $p = 0.001$, and mean ± SD free IS of 0.030 ± 0.006 *vs.* 0.016 ± 0.004 mg/dL, $p < 0.001$, respectively). It is noteworthy that there were no differences between CKD stage subgroups in terms of age, gender, race, BMI, diabetes status or time from CKD diagnosis (data not shown).

Table 2 shows selected bone histomorphometric parameters by CKD stage. Patients at CKD stages 2 and 3 presented remarkably low bone formation rate. In comparison with the latter subjects, patients at CKD stages 4 and 5 presented significantly higher osteoid volume, osteoblast surface, osteoclast surface, fibrosis volume and bone formation rate, and showed a trend toward lower mineralization lag time. There was no intergroup difference in trabecular bone volume. Importantly, none of the cohort members stained positively for aluminum at bone surface. Univariate regression analyses confirmed significant, negative, linear associations between the eGFR on one hand and the osteoid volume ($r^2 = 0.17$, $p = 0.004$), osteoblast surface ($r^2 = 0.26$, $p < 0.001$), fibrosis volume ($r^2 = 0.10$, $p = 0.03$) and bone formation rate ($r^2 = 0.21$, $p < 0.001$) on the other, as well as a positive association between the eGFR and the mineralization lag time ($r^2 = 0.09$, $p = 0.03$).

The associations between total IS serum levels and biochemical parameters related to mineral metabolism are shown in Table 3. A positive, linear association with phosphate, FGF-23 levels and iPTH levels and a negative association with 1,25 D levels were also noted.

Regarding the bone histomorphometric parameters, IS associated positively with the bone formation rate, osteoid volume, osteoblast surface and fibrosis volume (Table 4). Similar associations were observed with iPTH levels and the same histomorphometric parameters. Multivariate regression models (Table 5), revealed that the positive associations between IS serum levels on one hand, and the osteoblast surface and the bone fibrosis volume on the other were independent of the cohort's demographic characteristics and biochemical parameters related to mineral metabolism, such as iPTH, FGF-23, 25D and bicarbonate. A similar trend was observed for the bone formation rate.

Discussion

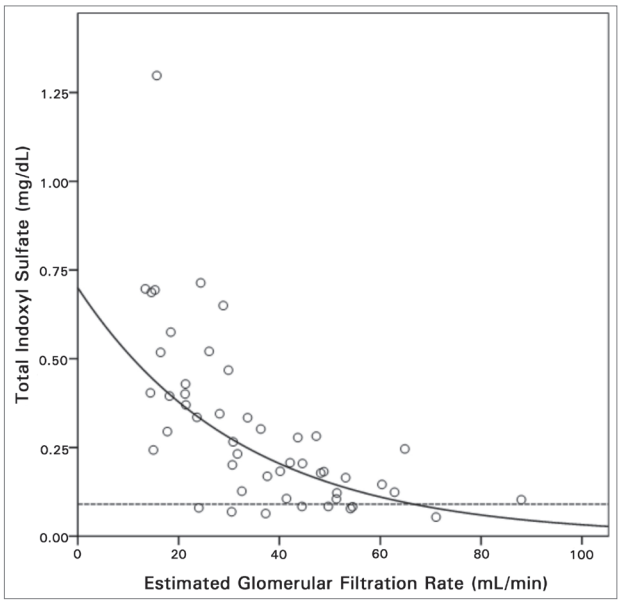
The present study suggests that under "real-life" conditions, IS levels are positively associated with the bone formation rate. This observation appears to contradict *in vitro* and animal studies, which reported an inhibitory effect of IS on bone formation, osteoblast-related gene

Table 1 Biochemical and hormonal characteristics of the study population, by CKD stage

	CKD stage		
	2 and 3 n = 29	4 and 5 n = 20	*p*
Bicarbonate (mM)	24.6 ± 2.9	22.6 ± 2.9	0.03
Ionized calcium (mM)	1.31 ± 0.04	1.28 ± 0.07	0.1
Phosphate (mg/dL)	3.5 ± 0.6	4.3 ± 0.6	< 0.001
Intact-PTH (pg/ml)	75 (41-99)	195 (99-366)	< 0.001
Total alkaline phosphatase (U/L)	113 (69-163)	118 (76-167)	0.6
bAP (U/L)	23 (17-28)	31 (21-46)	0.007
FGF-23 (pg/ml)	45.8 ± 30.1	95.8 ± 69.5	0.001
25 (OH) vitamin D (pg/ml)	33 ± 9	28 ± 10	0.05
1,25 $(OH)_2$ vitamin D (ng/dL)	38 (33-49)	33 (25-39)	0.02
Total indoxyl sulfate (mg/dL)	0.16 (0.09-0.22)	0.44 (0.35-0.68)	< 0.001
Free indoxyl sulfate (mg/dL)	0.028 (0.025-0.032)	0.041 (0.035-0.050)	0.003

Data are quoted as mean ± SD or, for variables with a non-Gaussian distribution, median (percentile 25^{th}-75^{th}). bAP: bone-specific alkaline phosphatase; FGF: Fibroblast growth factor; PTH: Parathyroid hormone.

Figure 1. Exponential association between serum total IS levels and the estimated glomerular filtration rate; $r^2 = 0.47$, $p < 0.001$, n = 49. The dotted line indicates the reference value in 20 healthy control subjects.



expression and both osteoblast and osteoclast cell viability.[9-11,21] However, the animal studies in question were performed in thyroparathyroidectomized uremic rats in which physiological plasma PTH concentrations were restored by infusion.[9,21] The inability to develop secondary hyperparathyroidism thus constitutes a limitation of this model. Although valid as a controlled, experimental data, the reported observations might be irreproducible under true clinical conditions, where impaired renal function is most frequently associated with supra normal increases in PTH secretion.[22]

Other important point of the present study is that we confirm previous studies that have suggested that low bone turnover might characterize ROD in early-stage CKD.[23] As suggested by observations in a murine model of CKD without secondary hyperparathyroidism[23] and a number of clinical studies,[4,24,25] the slight increase in PTH levels in early-stage CKD might not be sufficient to counterbalance certain bone anabolism-suppressive

Table 2 Bone histomorphometric parameters of the study population, by CKD stage

	CKD stage			
	2 and 3 n = 29	4 and 5 n = 20	*p*	Reference values
BV/TV (%)	17.02 ± 5.46	17.50 ± 5.67	0.9	20.95 ± 5.94
OV/BV (%)	0.95 (0.27-2.32)	2.31 (1.41-4.60)	0.003	2.18 ± 2.98
Ob.S/BS (%)	0.66 (0.16-1.34)	2.22 (1.05-4.35)	0.001	1.26 ± 2.44
ES/BS (%)	7.29 ± 6.72	8.72 ± 6.09	0.4	1.51 ± 1.27
Oc.S/BS (%)	0.22 (0.05-0.87)	0.70 (0.31-1.62)	0.015	0.01 ± 0.03
Fb.V (%)	0.6 (0-2)	1.5 (0.7-9.7)	0.02	< 0.5
BFR/BS ($\mu m^3/\mu m^2/d$)	0.001 (0.001-0.020)	0.010 (0.008-0.037)	0.004	0.04 ± 0.002
Mlt (d)	151 (34-655)	78 (31-181)	0.06	19 ± 7

Data are quoted as the mean ± SD or (for variables with a non-Gaussian distribution) the median (percentile 25-75). BV/TV: Trabecular bone volume/tissue volume; OV/BV: Osteoid volume/trabecular bone volume; Ob.S/BS: Osteoblast surface /bone surface; ES/BS: Eroded surface/bone surface; Oc.S/BS: Osteoclast surface/bone surface; Fb.V: Bone fibrosis volume; BFR/BS: Bone formation rate/bone surface; Mlt: Mineralization lag time.

Table 3 Univariate linear associations between serum total IS levels and mineral metabolism parameters

	R^2	β (95% CI)	*p*
Bicarbonate	0.13	-0.03 (-0.05- -0.007)	0.01
Ionized calcium	0.07	-1.1 (-2.2- -0.1)	0.1
Phosphate	0.22	0.16 (0.07-0.25)	0.001
intact-PTH	0.27	0.15 (0.08-0.23)	< 0.001
Total alkaline phosphatase	0.002	0.40 (-0.15-0.10)	0.7
Bone-specific alkaline phosphatase	0.25	0.15 (-0.02-0.32)	0.1
FGF-23	0.25	0.002 (0.001-0.003)	< 0.001
25 (OH) vitamin D	0.03	-0.004 (-0.01-0.003)	0.2
1,25 $(OH)_2$ vitamin D	0.16	-0.26 (-0.43- -0.10)	0.004

CI: Confidence interval; FGF: Fibroblast growth factor; PTH: Parathyroid hormone.

Table 4 Univariate linear associations between bone histomorphometric parameters on one hand and serum total IS and intact-PTH levels on the other

	Indoxyl sulfate			Intact-PTH		
	R^2	β (95% CI)	*p*	R^2	β (95% CI)	*p*
BV/TV	0.002	-0.002 (-0.01-0.01)	0.8	0.001	0.004 (-0.04-0.05)	0.8
OV/BV	0.15	0.07 (0.02-0.12)	0.006	0.33	0.34 (0.20-0.49)	< 0.001
Ob.S/BS	0.26	0.11 (0.05-0.17)	< 0.001	0.18	0.30 (0.10-0.50)	0.004
ES/BS	0.03	0.006 (-0.004-0.02)	0.2	0.03	0.02 (-0.16-0.06)	0.3
Oc.S/BS	0.04	0.04 (-0.02-0.10)	0.2	0.12	0.23 (0.04-0.42)	0.02
Fb.V	0.31	1.31 (0.73-1.90)	< 0.001	0.19	3.5 (1.3-5.6)	0.002
BFR/BS	0.16	0.06 (0.02-0.10)	0.004	0.14	0.19 (0.06-0.33)	0.007
MIt	0.04	-0.04 (-0.09-0.02)	0.2	< 0.001	-0.006 (-0.19-0.18)	0.9

CI: Confidence interval; the other abbreviations are as in Table 2.

Table 5 Multivariate linear regression models. Associations between serum IS levels and bone histomorphometric parameters

		β (95% CI)	*p*
OV/BV	Indoxyl sulfate	0.7 (-1.0-2.4)	0.4
Model's r^2 = 0.35[a]	intact-PTH	1.1 (0.4-1.7)	0.002
Ob.S/BS	Indoxyl sulfate	1.6 (0.1-3.1)	0.04
Model's r^2 = 0.34[b]			
Fb.V	Indoxyl sulfate	0.2 (0.1-3.2)	0.001
Model's r^2 = 0.44[c]	Ionized calcium	-0.7 (-1.2- -0.1)	0.01
BFR/BS	Indoxyl sulfate	1.8 (-0.2-3.8)	0.07
Model's r^2 = 0.24[d]	intact-PTH	0.5 (-0.03-1.2)	0.06

[a] Variables included in the model: indoxyl sulfate, ionized calcium, FGF-23, intact-PTH; [b] Variables included in the model: indoxyl sulfate, bicarbonate; FGF-23, 1,25-OH-vitamin D_3, intact-PTH; [c] Variables included in the model: indoxyl sulfate, age, ionized calcium, intact-PTH; [d] Variables included in the model: indoxyl sulfate, age, intact-PTH; Abbreviations are as in Table 2.

factors already present (such as gonadal hormone deficiency, diabetes and decreased calcitriol).[23,26] It is important to note that none of the patients enrolled in the present study had ever been treated with vitamin D analogues, calcium or aluminum-based phosphate binders or drugs associated with low bone turnover. Hence, in contrast to earlier reports in hemodialysis patients, possible iatrogenic effects of these drugs cannot be implicated in the lower bone turnover observed in this study.

One explanation for the apparently conflict between our present clinical observation and the preclinical work in the literature relates to the fact that higher IS levels may affect PTH secretion by either participating in skeletal resistance to PTH or by decreasing calcitriol synthesis/action. The hyperparathyroidism, commonly present in the uremic state would then clinically override the local inhibitory action of IS on bone turnover. There are at least two major arguments in favor of this hypothesis.

Firstly, it has been demonstrated that uremia is associated with down-regulation/desensitization of the PTH1R, that contributes to the skeletal resistance to PTH observed in the uremic setting.[27] Importantly, this condition is neither prevented nor corrected by parathyroidectomy.[28] It has subsequently been reported that factors other than PTH present in the uremic ultrafiltrate decrease PTH-stimulated cAMP generation in cultured osteoblast-like cells via a decrease in the levels of PTH1R mRNA.[29] More specifically, Nii-Kono *et al*. demonstrated that IS reduced PTH-induced cAMP production, PTH1R gene expression and cell viability in primary osteoblast cell cultures.[10] Thus, uremic toxins, and particularly IS, may be directly involved in the development of bone resistance to PTH. The latter condition may stimulate PTH synthesis aggravating the secondary hyperparathyroidism and leading to higher bone turnover in later CKD stages. In accordance with this hypothesis, our early-stage CKD patients, that

presented only slightly elevated iPTH levels but significantly elevated serum IS levels, had extremely low bone formation rate. This possibly reflects an initial, preponderant inhibitory action of IS on bone turnover prior to major elevation of PTH levels.

Secondly, calcitriol availability may be affected by IS. In addition to classical factors known to reduce calcitriol synthesis (i.e. decreased renal mass, hyperphosphatemia, increased FGF-23 levels and metabolic acidosis), phosphate-free uremic plasma has also been shown to directly inhibit 1α-hydroxylase activity and thus decrease calcitriol availability.[30] Calcitriol exerts its biologic action by binding to the nuclear vitamin D receptor (VDR). Under normal conditions, calcitriol suppresses PTH secretion by directly binding to VDRs in the parathyroid gland. Patel *et al.*[31] have elegantly demonstrated that VDRs isolated from rats with kidney failure and, subsequently, incubated with uremic ultrafiltrate presented a reduction in specific DNA binding capacity, which could not be explained by impaired VDR expression. The authors concluded that toxins present in the uremic ultrafiltrate interacted with the VDR to impair its DNA binding capacity within cells and could thus diminish the calcitriol response in kidney failure. Although further studies have identified a role for purines in this process,[32,33] it is likely that other compounds are involved. By studying subfractions of uremic plasma, Hsu *et al.*[32] further demonstrated that other substances, which included IS among them, could also interfere on calcitriol metabolism. Moreover, a preliminary study has shown that rats with kidney failure fed with a high-protein diet presented significantly lower calcitriol levels than animals fed a normal diet, in the absence of intergroup differences in phosphate intake and creatinine clearance.[32,34] Since higher protein intake results in greater IS levels,[35] this observation further suggests that IS has a negative effect on calcitriol synthesis. Our finding in the present cohort of a negative association between 1,25 D and IS serum levels corroborates to this hypothesis.

Our study's strong points include the first ever evaluation in a clinical setting of biochemical and hormonal factors related to mineral metabolism and bone histomorphometry and their association with IS, a protein-bound uremic toxin prototype, in asymptomatic, treatment-naïve patients at different pre-dialysis CKD stages. Limitations of our study include the small cohort size. Additionally, due to the inverse relationship between GFR and IS levels (as depicted in Figure 1), it is not possible to differentiate whether the observed association between this uremic toxin and bone histomorphometric parameters is independent of kidney function loss. Actually, the study's epidemiological, cross-sectional design does not enable cause-effect relationships to be evaluated. Hence, the present study should be considered as generating hypotheses, which should be tested in infusion studies in animals and then confirmed by interventional clinical trials.

Conclusions

The results of the present study demonstrated that the uremic toxin IS is positively associated with bone formation rate, osteoid volume, osteoblast surface and fibrosis volume in a CKD setting - possibly by increasing PTH secretion (via an increase in skeletal resistance to PTH or via calcitriol inhibition) - suggesting that IS may play a role in the pathogenesis or ROD in the pre-dialysis setting.

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