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SCIENTIFIC INVESTIGATIONS

# Effect of Mineral and Bone Metabolism on Restless Legs Syndrome in Hemodialysis Patients

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**Study Objectives:** Restless legs syndrome (RLS) is a highly prevalent sleep disease among patients on hemodialysis. The physiopathology is still unclear, and may be multifactorial. Because of the association between iron metabolism and chronic kidney disease-mineral and bone disorders (CKD-MBD), we hypothesized that both factors would be associated with RLS.

**Methods:** We have evaluated hemodialysis patients, in a face-to-face interview for the diagnosis and severity of RLS, as measured by the International Restless Legs Syndrome Study Group. Clinical, demographic, and biochemical characteristics were measured.

**Results:** Out of 101 adult patients included, RLS was found in 29 (28.7%). Adjusted multinomial regression analysis revealed that age older than 35 years, transferrin saturation less than 47%, serum ferritin level less than 700 ng/mL, hemoglobin level less than 9.8 g/dL, serum phosphate level higher than 5.2 mg/dL, FGF-23 higher than 2,000 RU/mL, and C-reactive protein less than 1.24 mg/dL were independently associated with RLS. RLS was classified as mild, moderate, severe, and very severe in 3.4%, 41.7%, 44.8%, and 10.1% of patients, respectively. Scores of severity correlated significantly with erythropoietin dose/kg/w ($p = 0.046$), phosphate ($p = 0.003$), and inversely with serum albumin ($p = 0.003$) and calcium ($p = 0.008$). Phosphate and 25(OH)-vitamin D correlated with transferrin saturation. Patients with severe/very severe symptoms were mostly women, presented with lower serum iron, ionic calcium, and serum albumin levels and higher levels of serum phosphate, and higher percentage of 25(OH)-vitamin D deficiency and levels of FGF-23 higher than 2,000 RU/mL than did those with mild/moderate symptoms.

**Conclusions:** CKD-MBD factors besides iron metabolism are associated with RLS in patients on hemodialysis, providing new insights into the understanding of RLS in this population.

**Keywords:** chronic kidney disease, hemodialysis, mineral bone disorder, parathyroid hormone, phosphate

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## INTRODUCTION

Restless legs syndrome (RLS) is a movement disorder of sleep that is a common complaint of patients on dialysis therapy. Several reports in the literature have demonstrated the association between RLS in patients on dialysis and a negative effect on quality of life[1–3] and even mortality.[4,5] Though the diagnosis is simple, based on a specific questionnaire, and not time-consuming, this disorder remains overlooked.

Albeit there have been recent developments in understanding the pathophysiology of RLS, it is still not completely elucidated and likely to be multifactorial. Daily dialysis does not seem to improve RLS, showing only modest effects, which suggest that offering a high dose of dialysis is not enough to correct RLS.[6] Factors such as iron deficiency and altered dopamine metabolism have already been implicated in the pathophysiology of RLS.[7–11] More recently, disorders of bone and mineral disorders in patients with chronic kidney disease-mineral and bone disorders (CKD-MBD) has come to the fore as a possible new factor since hyperphosphatemia[12] and vitamin D deficiency[13–15] were associated with RLS in uremic patients and in the general population. Reinforcing these results, our group has just demonstrated that parathyroidectomy for patients with secondary hyperparathyroidism in hemodialysis alleviates RLS.[16]

Whether RLS is associated with CKD-MBD is undetermined. Recently, a finding has alerted on this possible association, as iron metabolism, which is altered in RLS may be associated to CKD-MBD. It has been demonstrated that both absolute and functional iron deficiency regulate the production

### BRIEF SUMMARY

**Current Knowledge/Study Rationale:** Restless legs syndrome (RLS) is a sleep disorder with high prevalence among patients on hemodialysis, for which the underlying mechanism is still unknown. This current study evaluated the role of chronic kidney disease-mineral and bone disorders (CKD-MBD) on RLS among patients on hemodialysis.

**Study Impact:** This study provides a new perspective on the physiopathology of RLS among patients on hemodialysis by showing that: (a) Factors of iron metabolism are associated with CKD-MBD; (b) CKD-MBD factors such as phosphate and 25(OH)-vitamin D correlated with iron metabolism (transferrin saturation); and (c) RLS in patients on hemodialysis may be related to hyperphosphatemia, high levels of fibroblast growth factor-23 (FGF-23), and high levels of parathyroid hormone.

of fibroblast growth factor-23, (FGF-23),[17] an osteocyte-derived hormone. In addition, lower levels of ferritin was independently associated with elevated FGF-23 in neonates.[18] Furthermore, correction of anemia with certain iron preparations has proved to decrease phosphate levels.[19] Nevertheless, the association between iron metabolism and CKD-MBD has not been tested in the presence of RLS.

We hypothesized that patients on hemodialysis with RLS, in addition to the classic iron metabolism homeostasis, would have unfavorable markers of CKD-MBD. Therefore, the specific aim of this study was to verify whether serum levels of 25(OH)-vitamin D, calcium, phosphate, and FGF-23 would be associated with RLS, as measured by the International Restless Legs Syndrome Study Group (IRLSSG),[20,21] in a cohort of patients on hemodialysis, through a face-to-face interview.

## METHODS

### Patients

We evaluated patients selected from a dialysis center located in a medical school hospital (Hospital das Clinicas, Universidade de Sao Paulo). Of 102 patients on dialysis, 101 were enrolled, after providing written consent, based on the following inclusion/exclusion criteria: age 18 y or older, stably treated with hemodialysis for at least 3 mo, and no signs of acute infection or neoplasia. Only one patient was excluded due to infection.

### Evaluation of RLS

The IRLSSG essential criteria scores were used to evaluate RLS,[20,21] validated with the Portuguese language.[22] The same physician had administered all questionnaires. Essential diagnostic criteria for RLS included urge to move the legs, worse during rest or inactivity, relieved by activity such as walking, and worse in the evening or night. Only patients endorsing all four questions were considered to have RLS. Common symptoms usually found in patients on dialysis, such as pruritus, were not classified as RLS, because not all criteria were fulfilled with an isolated symptom. Patients with a diagnosis of RLS were asked to complete the International RLS Severity Scale (IRLS) questionnaire. The IRLS has been developed by the IRLSSG and is a 10-item scale that enables a well-validated and easily administered measurement of RLS severity. Patients are classified as having mild, moderate, severe, and very severe RLS according to scores 1–10, 11–20, 21–30, and 31–40, respectively.

### Demographic, Clinical, and Biochemical Characteristics

Demographic and clinical data were extracted from clinical charts and computer records. The following demographic and comorbidity characteristics were collected at study entry: age, sex, race, underlying cause of end-stage renal disease (ESRD), presence of diabetes mellitus, history of hypertension, history of kidney transplant, congestive heart failure, coronary artery disease, peripheral vascular disease, cerebrovascular disease, presence of malignancy, and liver disease. Hemodialysis characteristics were also accessed, such as dialysis vintage, vascular access, average of ultrafiltration volume in the past month, presence of residual renal function, and standard Kt/V according to Daugirdas formula.[23] Biochemical variables evaluated were evaluated according to recommended by commercial assays. Parathyroid hormone (PTH) was measured by chemiluminescence immunoassay (reference range = 11–65 pg/mL; Roche immunoassay analyzer, Roche Diagnostics, Germany), FGF-23 was measured by enzyme-linked immunosorbent assay (C-terminal FGF-23; relative risk = 55 ± 50 RU/mL; Immutopics, San Clemente, CA, USA), and 25(OH)-vitamin D was measured by chemiluminescent immunoassay (Diasorin, Italy). Vitamin D deficiency was defined as levels less than 15 ng/mL. Medications in use recorded were: erythropoietin, angiotensin-converting enzyme inhibitor/angiotensin receptor blocker, beta-blocker, calcium channel blockers, vasodilators, diuretics, and statins.

### Statistical Analysis

Comparisons between patients with and without RLS and between mild-moderate versus severe-very severe RLS were done with Student *t*-test or Kruskal-Wallis test for continuous variables and chi-square test for categorical variables, as appropriate. The Pearson or Spearman correlation coefficient examined relationships between single variables. Logistic regression was done with RLS scores severe/very severe as dependent variable, and independent variables selected from univariate ($p < 0.010$). A multinomial regression was also performed to investigate factors associated with RLS, adjusting for covariates. Each variable was entered according to the best cutoff to predict RLS, according to the receiver operating characteristic curve. The likelihood ratio statistic was used to select variables for entry into the model. With this approach, age older than 35 y, male or female sex, transferrin saturation less than 47%, serum ferritin level less than 700 ng/mL, hemoglobin level less than 9.8 g/dL, serum calcium less than 7.2 mg/dL, serum phosphate level higher than 5.2 mg/dL, 25 (OH)-vitamin D less than 30 ng/mL, FGF-23 less than 6,000 RU/ml, PTH level higher than 700 pg/mL, and C-reactive protein level less than 1.24 mg/dL were selected (all values of $p < 0.010$). Data are presented as mean ± standard deviation or median (25,75 percentiles) according to normal or abnormal distribution by applying the D'Agostino-Pearson omnibus test. A value of $p < 0.05$ was considered significant. Analyses were performed with the use of SPSS 21.0.1 (SPSS Inc., Chicago, IL, USA).

## RESULTS

**Table 1** shows the characteristics of 101 ESRD patients included in the study. Underlying diseases leading to ESRD were chronic glomerulonephritis in 42 patients (41.6%), diabetic nephropathy in 7 patients (6.9%%), hypertensive nephrosclerosis in 12 patients (11.9%), other diseases in 34 patients (33.7%), and unknown disease in 6 patients (5.9%). Patients were relatively young and diabetes was found in 20.8%. All patients were receiving adequate dialysis doses, as indicated by a percent reduction of urea level higher than 65%. Of 101

**Table 1**—Characteristics of patients according to restless legs syndrome presence or absence.

| Characteristics | Absence of RLS (n = 72) | Presence of RLS (n = 29) | p |
|---|---|---|---|
| Female sex, n (%) | 29 (40.2%) | 18 (62.1%) | 0.047 |
| Age, y | 45 ± 15 | 47 ± 18 | 0.428 |
| Comorbidities, n (%) | | | |
| Hypertension | 49 (68.1) | 23 (79.3) | 0.258 |
| Diabetes | 17 (23.8) | 4 (13.8) | 0.271 |
| Heart failure | 14 (19.4) | 3 (10.3) | 0.269 |
| Coronary disease | 15 (20.8) | 3 (10.3) | 0.213 |
| Stroke | 3 (4.2) | 2 (6.9) | 0.567 |
| Previous kidney transplant | 18 (25) | 7 (24.1) | 0.928 |
| Hemodialysis parameters | | | |
| Arteriovenous fistula, n % | 48 (67) | 21 (72) | 0.574 |
| Residual diuresis > 300 mL, n (%) | 24 (33) | 13 (45) | 0.641 |
| Dialysis vintage, mo | 70 (29,185) | 71 (31,180) | 0.677 |
| Standard Kt/V | 2.4 ± 0.5 | 2.1 ± 0.4 | 0.210 |
| Average UF/session, l | 2.6 ± 1.0 | 2.8 ± 0.8 | 0.298 |
| Biochemical values | | | |
| $\beta_2$ microglobulin, µg/mL | 25.6 ± 9.0 | 25.1 ± 6.4 | 0.787 |
| C-reactive protein, mg/dL | 1.27 ± 0.41 | 1.22 ± 0.73 | 0.703 |
| Hemoglobin, g/L | 11.1 ± 1.7 | 11.1 ± 1.8 | 0.767 |
| Serum iron, µg/dL | 75.4 ± 34.3 | 62.9 ± 23.8 | 0.092 |
| Transferrin saturation, % | 46.1 ± 28.7 | 31.8 ± 15.7 | 0.019 |
| Ferritin, ng/mL | 504 (340, 832) | 420 (293, 645) | 0.384 |
| Erythropoietin, dose/kg/w, UI | 118 (61, 210) | 107 (31, 254) | 0.707 |
| Ionized calcium (mg/dL) | 4.7 ± 0.3 | 4.6 ± 0.4 | 0.280 |
| Total calcium (mg/dL) | 8.9 ± 0.8 | 8.8 ± 0.9 | 0.799 |
| Serum phosphate (mg/dL) | 5.2 ± 1.7 | 6.0 ± 1.7 | 0.048 |
| Calcium × phosphate product | 46.7 ± 16.5 | 52.4 ± 14.8 | 0.088 |
| PTH, pg/mL | 305 (144, 546) | 529 (190, 1,017) | 0.054 |
| PTH > 500 pg/mL, n (%) | 20 (28) | 15 (52) | 0.025 |
| FGF-23, RU/mL | 1,520 (664, 7,619) | 1,706 (553, 3,667) | 0.685 |
| Alkaline phosphatase (U/L) | 92 (65, 135) | 107 (61, 164) | 0.855 |
| 25 (OH)-vitamin D, ng/mL | 30.5 ± 8.5 | 28.8 ± 10.2 | 0.388 |
| 25 (OH)-vitamin D < 30 ng/mL, n (%) | 30 (42) | 18 (62) | 0.063 |
| History of previous parathyroidectomy, n (%) | 17 (24) | 6 (21) | 0.751 |
| Predialysis urea, mg/dL | 161 ± 38 | 173 ± 38 | 0.123 |
| Serum magnesium, mg/dL | 2.3 ± 0.3 | 2.2 ± 0.4 | 0.125 |
| Serum albumin, g/dL | 4.0 ± 0.33 | 4.1 ± 0.4 | 0.568 |

Values are mean ± standard deviation unless indicated otherwise. FGF-23 = fibroblast growth factor 23, PTH = parathyroid syndrome, RLS = restless legs syndrome.



patients, 29 (28.7%) had RLS. Three patients were taking clonazepam and 5 patients were been treated with gabapentin. No patient was receiving any dopamine agonist treatment before study entry. Antihistamines were prescribed to five patients with and six patients without RLS ($p > 0.99$). Antidepressive drugs have been prescribed to eight patients with and eight patients without RLS ($p > 0.99$). Female sex, low transferrin saturation and a significant proportion of PTH levels higher than 500 ng/mL characterized patients with RLS. In addition, patients with RLS had a tendency toward low serum iron levels, high calcium × phosphate product, high levels of PTH and serum phosphate, and deficiency of 25-(OH)-vitamin D. Multinomial regression analysis is presented in **Table 2**, showing that age, serum ferritin, transferrin saturation, FGF-23, serum phosphate, and hemoglobin levels, and also C-reactive protein levels were independently associated with RLS, in a model adjusted for sex, serum calcium, and C-reactive protein.

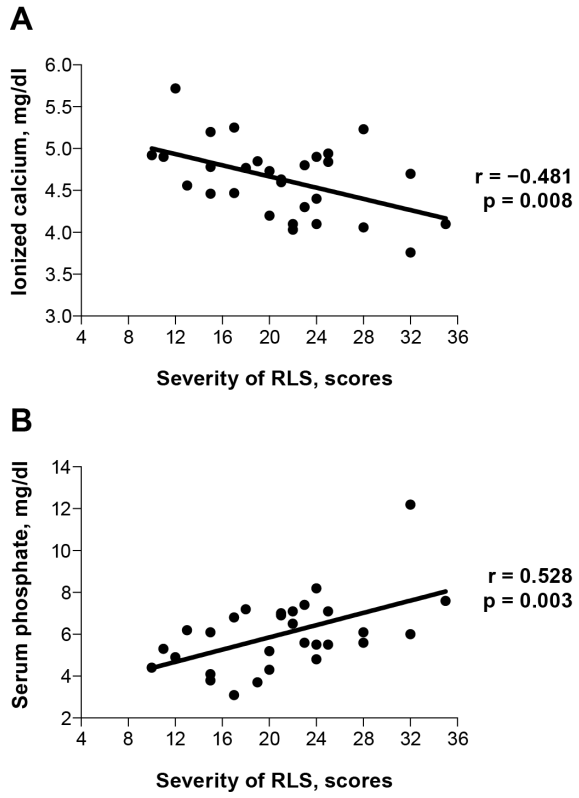
With respect to the severity of RLS, one patient was classified as mild (3.4%), 12 patients had moderate (41.7%), 13 patients had severe (44.8%), and 3 patients had very severe RLS (10.1%). Scores of RLS severity correlated with erythropoietin dose/kg/w ($r = 0.381$, $p = 0.046$), serum albumin ($r = -0.535$, $p = 0.003$), and had a tendency to correlate with FGF-23 ($r = 0.376$, $p = 0.053$). Some correlations with markers

**Table 2**—Multinomial logistic regressions predicting presence/absence of restless legs syndrome.

| Variable | OR | 95% Confidence Interval for OR | | p |
|---|---|---|---|---|
| | | Lower | Upper | |
| Sex (covariate) | | | | 0.735 |
| 25 (OH)-vitamin D < 30 ng/mL (covariate) | | | | 0.221 |
| Serum calcium < 8.8 mg/dL (covariate) | | | | 0.595 |
| Age older than 35 y | 2.69 | 1.46 | 3.91 | 0.007 |
| Transferrin saturation < 47% | 2.58 | 1.27 | 3.89 | 0.018 |
| Hemoglobin < 9.8 g/dL | 1.84 | 1.03 | 2.79 | 0.048 |
| Serum phosphate > 5.2 mg/dL | 1.84 | 1.04 | 2.63 | 0.040 |
| FGF-23 > 2,000 RU/mL | 3.16 | 2.13 | 4.18 | < 0.0001 |
| C-reactive protein > 1.24 mg/dL | 2.08 | 1.15 | 3.02 | 0.023 |
| Serum ferritin < 700 ng/mL | 2.12 | 1.14 | 3.10 | 0.025 |

FGF-23 = fibroblast growth factor-23, OR = odds ratio.

**Figure 1**—Relationship between scores of restless legs syndrome (RLS) severity and markers of mineral and bone metabolism such as ionized calcium **(A)**, and serum phosphate **(B)**.




of mineral metabolism such as ionized calcium and serum phosphate are shown in **Figure 1**.

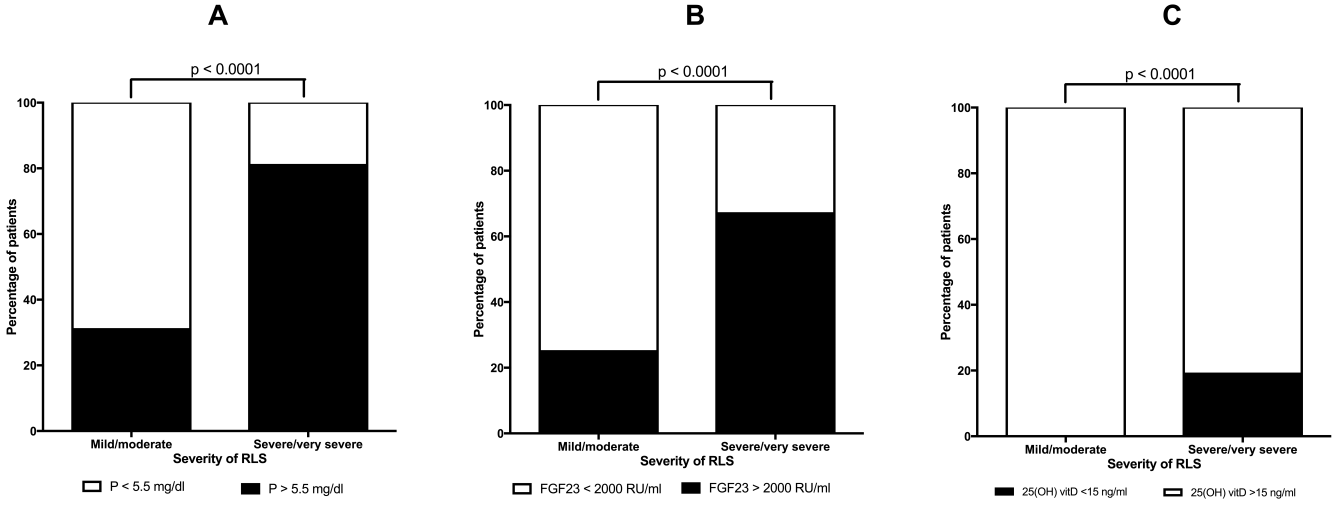
We found no correlation between iron metabolism (iron, transferrin saturation, and ferritin) and FGF-23. Phosphate had a tendency to correlate with transferrin saturation ($r = -0.168$, $p = 0.09$). 25(OH)-vitamin D correlated with transferrin saturation ($r = 0.267$, $p = 0.007$) and had a tendency to correlate with hemoglobin ($r = 0.192$, $p = 0.058$).

Patients were then divided into two groups according to the severity of RLS: mild/moderate (n = 13) and severe/very severe (n = 16). When compared with patients with mild/moderate RLS, those with severe/very severe symptoms were mostly women (81.3% vs. 38.5%, $p = 0.018$), had lower levels of serum iron (55.1 ± 20.2 vs. 72.5 ± 25.2 µg/dL, $p = 0.048$), ionized calcium (4.5 ± 0.4 vs. 4.8 ± 0.4 mg/dL, $p = 0.025$), and serum albumin (3.9 ± 0.3 vs. 4.3 ± 0.3g/L, $p = 0.005$), and also a tendency toward lower 25 (OH)-vitamin D levels (25.9 ± 10.2 vs. 32.3 ± 9.4 ng/mL, $p = 0.096$). Higher levels of serum phosphate (6.8 ± 1.7 vs. 5.0 ± 1.3 mg/dL, $p = 0.004$) and calcium × phosphate product (58.8 ± 14.1 vs. 44.6 ± 11.9 mg/dL, $p = 0.008$) were also seen in these patients. C-terminal FGF-23 (cFGF-23) levels ranged from 141 to 5,007 RU/mL (median 1,203 RU/mL) in patients with mild/moderate RLS and from 114 to 4,700 RU/mL (median 3,237 RU/mL) in patients with severe/very severe RLS, which was not different ($p = 0.256$). Using categorical variables, we found that patients with severe and very severe RLS also presented a significantly higher percentage of hyperphosphatemia (81.3% vs. 30.8%), 25(OH)-vitamin D deficiency (18.8% vs. zero) and also higher percentage of patients with FGF-23 above 2,000 RU/mL (67% vs. 25%, $p = 0.031$), as illustrated in **Figure 2**. In a multiple logistic regression model (Forward Stepwise - Likelihood Ratio), hyperphosphatemia, FGF-23 higher than 2,000 RU/mL, and female sex were independently related to RLS severe/very severe (all values of $p < 0.05$). In addition to these variables, this model has included levels of serum iron, ionized calcium, and albumin, and vitamin D deficiency as independent variables, which was not significant.

## DISCUSSION

In this study, we have confirmed that more than one-quarter of the studied population, even when composed of relatively younger individuals on hemodialysis, was affected by RLS. To our knowledge, this was the first attempt to correlate iron homeostasis and CKD-MDB factors with RLS. Although with a relatively small sample size, we demonstrated that RLS is

**Figure 2**—Differences in the percentage of hyperphosphatemia **(A)**, levels of fibroblast growth factor-23 (FGF-23) **(B)**, and 25(OH)-vitamin D deficiency **(C)** when comparing patients with severe/very severe and those with mild/moderate restless legs syndrome (RLS) symptoms.




associated with classic factors of anemia and also with some CKD-MBD markers such as high levels of phosphate, FGF-23 levels higher than 2,000 RU/mL, and deficiency of 25(OH)-vitamin D. Furthermore, we have also demonstrated that some of these CKD-MBD factors correlated with iron metabolism parameters.

The link between iron metabolism and dopamine neurotransmission has been well established, as iron deficiency causes a decrease in the density of dopamine D2 receptor in the striatum and nucleus accumbens,[9] accompanied by a reduction in dopamine transporter density.[8] This mechanism seems to be similar in patients on dialysis, because iron deficiency is usually reported to be associated with RLS.[1,11,24] The treatment of RLS comprises the supplementation of iron and the use of dopaminergic agonists.[24] The effect of iron supplementation, however, on patients on dialysis seems not to be sustainable.[25] In addition, the risk of iron overload and bone-related disease in patients on dialysis[26] precludes the widespread administration of iron beyond the recommended guidelines. Relative iron sufficiency was a characteristic of our patients, and half of them had serum iron levels higher than 66 µg/dL, transferrin saturation higher than 32%, and ferritin levels higher than 503 ng/mL. Despite this, 28% presented with RLS. This finding indicates a multifactorial physiopathology of RLS in patients on dialysis.

In individuals with normal renal function, iron deficiency can stimulate the production and the cleavage of the intact FGF-23, generating the c-terminal fraction.[19] In CKD, the cleavage is defective, so that practically all of the FGF is intact. Our hypothesis was that iron deficiency, even if relative, would stimulate the synthesis of FGF-23. High levels of FGF-23 would have a direct role in the pathogenesis of RLS, or would instead be only a marker of this sleep disease. We failed to demonstrated any correlation between FGF-23 and factors from iron metabolism, which is consistent with a previous study in neonates.[18] Both studies probably did not find such an association for the same reason: the iron sufficiency of the studied populations. In addition to this possible association between RLS and FGF-23, it must be stressed that FGF-23 levels are markedly elevated in patients on dialysis and are associated with hyperphosphatemia.[27] FGF-23 is a stable biomarker of disordered phosphate metabolism, and for this reason may also be associated with RLS. Nevertheless, we have found a correlation between some other markers of mineral and bone metabolism and iron metabolism. Of note, patients with RLS had vitamin D deficiency, high levels of phosphate, a high percentage of FGF-23 > 2,000 RU/mL, and also high levels of PTH that identified either the presence or the severity of RLS. Taken together, these data demonstrated that RLS is associated with iron metabolism and mineral and bone metabolism, which, in turn, are interconnected. Additional research of a larger number of patients on hemodialysis is warranted to evaluate this mechanism.

The literature has described the association between inflammatory markers with RLS in patients on hemodialysis.[28] Although we have found no correlation between RLS severity scores and C-reactive protein ($r = -0.094$, $p = 0.366$), this inflammatory marker was independently associated with RLS in a multinomial regression analysis.

Our study is subject to several limitations: first, it was a small sample size in a one-center study. Second, our population presented sufficiency of iron, making it difficult to find an association between iron and mineral metabolism. Third, we have no objective data on sleep to assess the effect of RLS on sleep quality, although this was not the main objective of the current study. Finally, the cross-sectional nature and the observational design do not allow establishing a cause-effect relationship. However, the strength of the current study lies in demonstrating, for the first time, the association between CKD-MBD and iron metabolism in a scenario of RLS, among patients on hemodialysis. If confirmed, our results may help the management of RLS in patients on dialysis, warning to the role of CKD-MBD.

In summary, our findings generally support previous studies on the relationship between iron metabolism and RLS, and additionally provide possible new insights into the understanding of this sleep disorder in patients on hemodialysis. Future research should verify if the treatment of hyperparathyroidism by either medication or parathyroidectomy might alleviate RLS.

## ABBREVIATIONS

CKD-MBD, chronic kidney disease-mineral and bone disorders
ESRD, end-stage renal disease
FGF-23, fibroblast growth factor-23
IRLS, International RLS Severity Scale
IRLSSG, International Restless Legs Syndrome Study Group
PTH, parathyroid hormone
RLS, restless legs syndrome

## REFERENCES


1. Giannaki CD, Hadjigeorgiou GM, Karatzaferi C, Pantzaris MC, Stefanidis I, Sakkas GK. Epidemiology, impact, and treatment options of restless legs syndrome in end-stage renal disease patients: an evidence-based review. *Kidney Int.* 2014;85(6):1275–1282.
2. Mucsi I, Molnar MZ, Ambrus C, et al. Restless legs syndrome, insomnia and quality of life in patients on maintenance dialysis. *Nephrol Dial Transplant.* 2005;20(3):571–577.
3. Lee J, Nicholl DD, Ahmed SB, et al. The prevalence of restless legs syndrome across the full spectrum of kidney disease. *J Clin Sleep Med.* 2013;9(5):455–459.
4. Lin CH, Sy HN, Chang HW, et al. Restless legs syndrome is associated with cardio/cerebrovascular events and mortality in end-stage renal disease. *Eur J Neurol.* 2015;22(1):142–149.
5. Gholamrezaei A, Amra B, Mortazavi M. Cardiovascular risk and mortality in end-stage renal disease patients with restless legs syndrome; need for further investigation and looking for underlying mechanisms. *Sleep Med.* 2013;14(4):385–386.
6. Hanly PJ, Gabor JY, Chan C, Pierratos A. Daytime sleepiness in patients with CRF: impact of nocturnal hemodialysis. *Am J Kidney Dis.* 2003;41(2):403–410.
7. Beard J, Erikson KM, Jones BC. Neonatal iron deficiency results in irreversible changes in dopamine function in rats. *J Nutr.* 2003;133(4):1174–1179.
8. Erikson KM, Jones BC, Beard JL. Iron deficiency alters dopamine transporter functioning in rat striatum. *J Nutr.* 2000;130(11):2831–2837.
9. Erikson KM, Jones BC, Hess EJ, Zhang Q, Beard JL. Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. *Pharmacol Biochem Behav.* 2001;69(3-4):409–418.
10. Merlino G, Lorenzut S, Gigli GL et al. A case-control study on restless legs syndrome in nondialyzed patients with chronic renal failure. *Mov Disord.* 2010;25(8):1019–1025.
11. Novak M, Winkelman JW, Unruh M. Restless legs syndrome in patients with chronic kidney disease. *Semin Nephrol.* 2015;35(4):347–358.
12. Takaki J, Nishi T, Nangaku M, et al. Clinical and psychological aspects of restless legs syndrome in uremic patients on hemodialysis. *Am J Kidney Dis.* 2003;41(4):833–839.
13. Oran M, Unsal C, Albayrak Y, et al. Possible association between vitamin D deficiency and restless legs syndrome. *Neuropsychiatr Dis Treat.* 2014;10:953–958.
14. Wali S, Shukr A, Boudal A, Alsaiari A, Krayem A. The effect of vitamin D supplements on the severity of restless legs syndrome. *Sleep Breath.* 2015;19(2):579–583.
15. Balaban H, Yildiz OK, Cil G, et al. Serum 25-hydroxyvitamin D levels in restless legs syndrome patients. *Sleep Med.* 2012;13(7):953–957.
16. Santos RS, Coelho FM, Silva BC, et al. Parathyroidectomy Improves Restless Leg Syndrome in Patients on Hemodialysis. *PLoS One.* 2016;11(5):e0155835.
17. David V, Martin A, Isakova T, et al. Inflammation and functional iron deficiency regulate fibroblast growth factor 23 production. *Kidney Int.* 2016;89(1):135–146.
18. Ali FN, Josefson J, Mendez AJ, Mestan K, Wolf M. Cord blood ferritin and fibroblast growth factor-23 levels in neonates. *J Clin Endocrinol Metab.* 2016;101(4):1673–1679.
19. Wolf M, Koch TA, Bregman DB. Effects of iron deficiency anemia and its treatment on fibroblast growth factor 23 and phosphate homeostasis in women. *J Bone Miner Res.* 2013;28(8):1793–1803.
20. Allen RP, Picchietti DL, Garcia-Borreguero D, et al. Restless legs syndrome/Willis-Ekbom disease diagnostic criteria: updated International Restless Legs Syndrome Study Group (IRLSSG) consensus criteria--history, rationale, description, and significance. *Sleep Med.* 2014;15(8):860–873.
21. International Restless Legs Syndrome Study Group. 2012 Revised IRLSSG Diagnostic Criteria for RLS. International Restless Legs Syndrome Study Group Web site. http://irlssg.org/diagnostic-criteria/. Accessed September 23, 2016.
22. Masuko AH, Carvalho LB, Machado MA, Morais JF, Prado LB, Prado GF. Translation and validation into the Brazilian Portuguese of the restless legs syndrome rating scale of the International Restless Legs Syndrome Study Group. *Arq Neuropsiquiatr.* 2008;66(4):832–836.
23. Daugirdas JT. Dialysis adequacy and kinetics. *Curr Opin Nephrol Hypertens.* 2000;9(6):599–605.
24. Daubian-Nose P, Frank MK, Esteves AM. Sleep disorders: a review of the interface between restless legs syndrome and iron metabolism. *Sleep Sci.* 2014;7(4):234–237.
25. Sloand JA, Shelly MA, Feigin A, Bernstein P, Monk RD. A double-blind, placebo-controlled trial of intravenous iron dextran therapy in patients with ESRD and restless legs syndrome. *Am J Kidney Dis.* 2004;43(4):663–670.
26. Rocha LA, Barreto DV, Barreto FC, et al. Serum ferritin level remains a reliable marker of bone marrow iron stores evaluated by histomorphometry in hemodialysis patients. *Clin J Am Soc Nephrol.* 2009;4(1):105–109.
27. Isakova T, Xie H, Barchi-Chung A, et al. Fibroblast growth factor 23 in patients undergoing peritoneal dialysis. *Clin J Am Soc Nephrol.* 2011;6(11):2688–2695.
28. Higuchi T, Abe M, Mizuno M, et al. Association of restless legs syndrome with oxidative stress and inflammation in patients undergoing hemodialysis. *Sleep Med.* 2015;16(8):941–948.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