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Relation of serum hepcidin levels and restless legs syndrome in chronic hemodialysis patients

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Abstract

Background Iron deficiency anemia, pregnancy, and end-stage renal disease (ESRD) are common causes of secondary restless legs syndrome (RLS). Serum ferritin is considered the most specific test associated with the total amount of body iron stores. However, due to the increase of serum ferritin secondary to inflammation in chronic hemodialysis (HD) patients, serum ferritin test results do not fully reflect decreased iron stores in these patients. The present study evaluates the serum hepcidin levels, as the main regulator of iron metabolism, and its relationship with RLS in chronic HD patients.

Methods The present cross-sectional study involved 72 patients (36 with and 36 without RLS) who received chronic HD treatment between April 2014 and April 2015. Demographic and biochemical data were evaluated in all patients, and statistical analyses were performed.

Results The mean age and mean dialysis vintage of all patients (56% women) included in the study were 65.3 ± 11.6 years and 41.5 ± 36.5 months, respectively. Serum hepcidin, hemoglobin A1C (HbA1C), and ferritin levels were significantly higher in patients with RLS ($p = 0.001$, $p = 0.032$, $p = 0.042$, respectively). In addition, a positive correlation was found between International Restless Legs Syndrome Study Group severity scale score and serum hepcidin levels, HbA1C, and ferritin ($r = 0.387, p = 0.001$; $r = 0.426, p = 0.034$; $r = 0.240, p = 0.046$, respectively). A multivariate linear regression analysis revealed hepcidin and HbA1C to be independently associated with the presence of RLS.

Conclusion A significant relationship was detected between RLS and increased serum hepcidin levels in chronic HD patients, and uncontrolled diabetes was noted to contribute to this association.

Keywords Restless legs syndrome · Hemodialysis · End-stage renal disease · Hepcidin · Ferritin · Inflammation

Introduction

Restless legs syndrome (RLS) is a common neurological disease that disrupts the quality of life and sleep, with yet unclear pathophysiology. Current studies support that dopaminergic dysfunction and the change of iron balance control as a result of a decrease in iron levels in the central nervous system play an important role in pathophysiology [1, 2].

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In healthy individuals, there is a recycling process in the reticuloendothelial system that meets about 90% of the body's daily iron needs. The remaining 10% of this need is met through intestinal absorption. When needed, iron is rapidly released into the circulating transferrin or stored in ferritin when it is excessive. Increased transferrin-bound iron and high iron stores stimulate upregulation of hepcidin, which lowers circulating iron levels by inhibiting iron export from duodenal enterocytes, splenic macrophages, and hepatocytes. In the presence of reduced iron levels in the circulation, hepcidin production is inhibited. Ferritin is an acute phase protein and serum ferritin levels can be profoundly affected by the presence of inflammation. In inflammatory conditions, increased serum ferritin levels cannot accurately reflect the presence of iron, and the assessment of iron status becomes more complicated. In addition, in response to inflammatory cytokines such as IL-6, hepcidin production increases, and the availability of iron is restricted to bind to the transferrin [3].

RLS is categorized into two main groups, being primary or secondary, although people with secondary RLS are also suggested to have a genetic basis for the disorder, like patients with primary RLS, and symptoms of RLS have been found to increase with the increased load of comorbid diseases [4]. Two different RLS phenotypes have been defined as early- and late-onset RLS according to the symptom onset age. Patients with early onset have been shown to have a significant genetic factor. Late-onset RLS patients are considered to be a mixture of secondary RLS disease and primary RLS patients exacerbated by secondary factors [2]. The findings of central iron deficiency were demonstrated in detail by magnetic resonance imaging, autopsy, and cerebrospinal fluid (CSF) evaluation in patients with primary RLS [5, 6]. It is highlighted that patients with late-onset RLS, which constitute the majority of secondary RLS patients, have a lower serum ferritin level than patients with early onset. Therefore, not only the brain iron status but also the body iron status is important for these patients [2]. In a study of primary RLS patients, there was no significant increase in serum ferritin levels compared with the control group, while an increase in hepcidin level was shown to be significant [7]. In another study, pro-hepcidin levels were found to be increased in the neuromelanin cells in the brain parenchyma [5].

Pregnancy, iron deficiency anemia, and end-stage renal disease (ESRD) are among the causes of secondary RLS in which iron deficiency is commonly seen [2, 8]. Iron deficiency anemia is a frequent problem in chronic hemodialysis (HD) patients. Approximately 24–85% of patients with chronic renal failure (CRF) are iron-deficient (highest incidence with more severe CRF). Hepcidin levels increase progressively with the severity of CRF and HD patients with a six- to nine-fold increase of hepcidin [3]. Malnutrition, inflammation, and blood loss during HD sessions are among the many causes of this condition, although increased serum hepcidin levels can also contribute to the development of anemia by blocking the absorption of iron from the intestines [1]. RLS is reported to be common in these patients due to such comorbid conditions as disorders of the iron metabolism, systemic inflammation, peripheral neuropathy, diabetes mellitus (DM), dialysis-related factors, and calcium/phosphate imbalance [1, 8]. In chronic HD patients, iron absorption and use are impaired due to the increased serum hepcidin level, and the serum ferritin level that increases secondary to inflammation cannot fully reflect the deteriorated condition of iron stores. In the present study, we measured the serum hepcidin and ferritin levels in chronic HD patients to evaluate the relation between the laboratory parameters, including serum hepcidin and ferritin levels, and RLS.

Methods

Patients

This prospective cross-sectional study assesses the neurological symptoms of RLS in 72 HD patients at baseline, with subsequent observations of major clinical and laboratory parameters. The diagnostic criteria of RLS were retrieved from the International RLS Study Group (IRLSSG) [9]. The IRLSSG rating scale (IRLS) was used to determine RLS severity [10]. Patients who had been undergoing chronic HD treatment for at least 6 months and who were aged ≥ 18 years were included in the study. The exclusion criteria were recent hospitalization, major surgery, obvious infections, or inflammatory disease within the last 3 months, end-stage liver disease, malignancies, drug addiction, neurological diseases, and malabsorption syndromes. None of the patients took drugs affecting the central nervous system or any other medication known to affect sleep or movement. All patients received 4 h of conventional HD three times a week with standard bicarbonate-containing dialysate and biocompatible low-flux HD membranes. The blood flow rates ranged from 300 to 350 mL/min, and the dialysate flow rate was kept constant at 500 mL/min. The delivered dose of dialysis (Kt/V) was calculated using the method described by Daugirdas. The study protocol was approved by the local ethics committee, and all patients provided written informed consent.

Demographics and laboratory analysis

The initial assessment of all patients included demographics and other parameters [age, gender, body mass index (BMI), duration of HD, presence of DM, hypertension (HT), and cardiovascular disease duration of RLS, IRLS score, and polyneuropathy], biochemical data [fasting plasma glucose (FPG), hemoglobin A1C (HbA1C), serum urea, creatinine, uric acid, sodium, potassium, hemoglobin, calcium, phosphorus, intact parathyroid hormone (i-PTH), albumin level, iron status (serum iron, total iron-binding capacity (TIBC), ferritin, and transferrin saturation), and inflammation markers (C-reactive protein [CRP] and interleukin-6 [IL-6])]. Fasting blood samples for biochemical analysis were obtained before the midweek HD sessions, and analyses were performed using standard auto analyzers at 08 am. BMI was calculated (kg/m^2) using weights and heights that were measured 15–30 min after the end of the midweek HD sessions. All patients underwent electromyography to determine the presence of polyneuropathy.

Since hepcidin and IL-6 are not routinely analyzed at the hospital, the method for the calculation of serum hepcidin and IL-6 levels was as follows. Blood samples were drawn after overnight fasting and centrifuged within 1 h at 2500g for 10 min. The isolated serum was stored at − 80 °C. Serum

concentrations of human IL-6 were determined in duplicate through a commercial enzyme-linked immunosorbent assay (ELISA) test (eBioscience, Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions. The intra-assay and inter-assay coefficients of variation (CV) were 3.4% and 5.2%, respectively. The sensitivity was calculated as 0.92 pg/mL. The serum levels of human hepcidin were quantified by an ELISA using commercially available matched antibodies (Uscn Life Science İnc. Wuhan, China). The intra-assay and inter-assay CV were < 10.0% and < 12.0%, respectively. Sensitivity was calculated as 0.0235 ng/mL.

Statistical analysis

SPSS 20.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data were presented as mean ± SD. The normality of the distribution of the measured data was assessed with a Kolmogorov–Smirnov test. Mann–Whitney *U* and Chi-square tests were used for the comparison of numerical values with non-normal distribution and the evaluation of categorical data, respectively. The correlation between the IRLS score and all parameters was evaluated with Spearman's correlation analysis. The factors found to be associated with the presence of RLS in univariate analysis were included in the multivariate linear regression analysis using the Enter method. The cut-off value of serum hepcidin predicting the presence of RLS was determined using a ROC analysis (sensitivity, specificity, and positive and negative predictive values). A p value of < 0.05 was accepted as statistically significant.

Results

The demographic data and laboratory analysis results of the 72 patients with HD are shown in Table 1. Of the total, 36 patients (50%) were diagnosed with RLS. The mean IRLS score was 19.5 ± 7.2. The average age of all patients was 65.3 ± 11.6 years with a male/female ratio of 32/40. No age, gender, or BMI differences were noted between those HD patients with and those without RLS. The duration of HD and the frequency of DM, HT, cardiovascular disease, and polyneuropathy were also similar in the groups.

Among the laboratory parameters, serum hepcidin, HbA1C, and ferritin levels were found to be significantly higher in HD patients with RLS than in HD patients without RLS (p = 0.001, p = 0.032, and p = 0.042, respectively). No significant differences were identified in the other parameters.

The correlation of all parameters with the IRLS score was evaluated using a bivariate Spearman's correlation test, and a positive correlation was found between serum hepcidin, HbA1C, and ferritin levels and IRLS score (r = 0.387, p = 0.001; r = 0.426, p = 0.034; r = 0.240, and p = 0.046, respectively) (see Table 2, Fig. 1). A multivariate linear regression analysis was performed on the factors determined to have a relation with the presence of RLS in univariate analysis, and hepcidin and HbA1C were still found to have an independent relation with the presence of RLS (95% confidence interval [CI]:0.000–0.006, p = 0.035; 95% CI: 0.012–0.293, p = 0.035, respectively) (Table 3). A ROC analysis was performed to determine the relation between hepcidin level and RLS. The cut-off value of serum hepcidin predicting the presence of RLS was found to be 165.80 ng/mL (sensitivity: 61.1%, specificity: 77.8%, and positive predictive value: 2.77, negative predictive value: 0.49) (area under the curve [AUC]: 726, 95% CI: 0.607–0.846, p = 0.001) (see Fig. 2).

Discussion

It was found in the present study that serum hepcidin levels were significantly higher in patients with RLS when compared with those without RLS among the chronic HD patients. Uncontrolled diabetes was found to contribute to an increased hepcidin level, and an increase in serum hepcidin level was found to be positively correlated with symptom severity in RLS.

A special etiology is yet to be found to explain the clinical findings, although studies into the pathophysiology of RLS have been continuing for many years. Many years of genetic research have led to the genetic heterogeneity of the disease being revealed, and RLS to be considered a prevalent, complex genetic disease [11]. ESRD is among the common causes of secondary RLS. RLS is encountered in almost one-quarter of all patients with renal failure, while this rate is one-third in patients with ESRD [12]. The pathogenic mechanisms responsible for RLS associated with renal failure are still unknown. Functional impairment of the dopaminergic system and decreased iron stores in some specific regions of the brain are a widely accepted hypothesis for idiopathic RLS. It has been suggested in studies reporting that treatment with dopamine agonists was also beneficial in patients with RLS associated with renal failure that dopaminergic dysfunction may play role in the pathophysiology of RLS associated with renal failure [13].

Frequent iron deficiency and the high prevalence of RLS in patients with ESRD support the role of iron deficiency in the pathophysiology of the disorder in these patients [2, 13, 14]. Another finding supporting this relation is that the utility of iron is increased, and symptoms of RLS are improved following renal transplantations in HD patients [1, 15]. Calcium/phosphate imbalance has also been reported to possibly play a role in the pathophysiology of the disorder [2, 13, 14]. Polyneuropathy is among the common causes of secondary RLS; however, the relation between polyneuropathy and RLS has not been completely confirmed in HD patients, in whom polyneuropathy is frequently detected [1, 4, 13].

Table 1 Characteristics of HD patients with and without RLS

	All patients ($n = 72$)	RLS (+) ($n = 36$)	RLS (−) ($n = 36$)	p
Demographics				
Age (year)	65.3 ± 11.6	64.2 ± 11.2	66.5 ± 12.0	0.437
Gender (male/female)	32/40	15/21	17/19	0.813
Body mass index (kg/m^2)	27.7 ± 4.5	28.23.3	27.1 ± 5.5	0.306
Duration of HD (months)	41.536.5	42.9 ± 40.5	40.1 ± 32.6	0.652
DM (n, %)	40 (55.5%)	20 (55.5%)	20 (55.5%)	1
HT	55 (76.4%)	28 (77.8%)	27 (75%)	0.782
Cardiovascular disease	17 (23.6%)	8 (22.2%)	9 (25%)	0.465
Duration of RLS (year)	---	5.1 ± 4.0	---	---
IRLS score	---	19.5 ± 7.2	---	---
Polyneuropathy (n, %)	43 (59.7%)	24 (66.7%)	19 (52.8%)	0.336
Delivered dose of dialysis				
spKt/V	1.52 ± 0.42	1.54 ± 0.36	1.48 ± 0.38	0.384
Laboratory				
FPG (mg/dL)	135.2 ± 77.0	139 ± 78.3	131.6 ± 76.7	0.388
HbA1C (%)	7.4 ± 1.4	8.0 ± 1.4	6.8 ± 1.0	0.032
Urea (mg/dL)	112.4 ± 57.0	107.2 ± 60.3	117.2 ± 54.1	0.188
Creatinine (mg/dL)	8.2 ± 2.3	8.5 ± 2.1	8.0 ± 2.5	0.272
Uric acid (mmol/L)	6.3 ± 1.2	6.5 ± 1.3	6.1 ± 1.1	0.209
Sodium (mmol/L)	137.3 ± 2.9	137.2 ± 2.7	137.3 ± 3.1	0.786
Potassium (meq/L)	5.4 ± 0.7	5.5 ± 0.7	5.4 ± 0.6	0.716
Hemoglobin (g/dL)	11.1 ± 1.3	11.1 ± 1.4	11.0 ± 1.1	0.506
Calcium (mg/dL)	8.8 ± 0.7	8.8 ± 0.6	8.9 ± 0.7	0.368
Phosphorus (mg/dL)	5.5 ± 1.1	5.6 ± 1.1	5.4 ± 1.2	0.381
CaXP	49.2 ± 9.9	49.8 ± 9.1	48.6 ± 10.7	0.388
Intact PTH (pg/mL)	314.5 ± 250.2	316.8 ± 243.4	312.3 ± 259.9	0.934
Albumin (g/L)	3.7 ± 0.3	3.8 ± 0.4	3.6 ± 0.3	0.080
Iron (μg/dL)	61.6 ± 26.0	67.1 ± 28.9	56.5 ± 22.2	0.150
TIBC (g/L)	189.7 ± 58.8	193.5 ± 63.9	186.2 ± 54.3	0.292
TSAT (%)	37.4 ± 23.4	42.7 ± 29.5	32.5 ± 14.7	0.440
Ferritin (ng/mL)	528.4 ± 390.5	625.6 ± 439.4	436.7 ± 317.3	0.042
CRP (mg/dL)	1.1 ± 1.3	0.8 ± 0.8	1.4 ± 1.5	0.230
IL-6 (pg/mL)	8.9 ± 9.8	7.7 ± 7.5	10.1 ± 11.7	0.424
Hepcidin (ng/mL)	155.9 ± 87.3	188.4 ± 82.7	123.4 ± 80.4	0.001

Data presented as mean ± standard deviation

p *p* value, *HD* hemodialysis, *RLS* restless leg syndrome, *DM* diabetes mellitus, *HT* hypertension, *IRLS* RLS study group rating scale, *spKt/V* single pool *Kt/V*, *FPG* fasting plasma glucose, *HbA1C* hemoglobin A1C, *CaXP* calcium and serum phosphorus, *PTH* parathormone, *TIBC* total iron-binding capacity, *TSAT* transferrin saturation, *CRP* C-reactive protein, *IL-6* interleukin 6

Even though erythropoietin deficiency is the leading cause of the development of anemia in chronic HD patients, iron deficiency anemia is also common in this group of patients. Malnutrition, chronic blood loss during HD sessions, regular collection of blood for laboratory tests, platelet dysfunction, and occult intestinal bleeding secondary to uremic enteropathy are among the causes of this condition [1, 16]. Iron levels and iron deficiency anemia of these patients are evaluated using routinely performed tests to ascertain serum iron, transferrin saturation (TSAT), and ferritin levels. Serum ferritin levels, as a component of an acute phase response due to chronic inflammation, are also increased in patients with CRF. As such, the serum ferritin level alone in such patients does not exclude iron deficiency. An evaluation of TSAT is generally needed as an additional test [3]. Both serum ferritin and TSAT are readily available and cost-effective tests.

Hepcidin is the main hormone regulating the iron levels in the human body, although it is not routinely measured in

Table 2 Bivariate correlation analysis of IRLS score and other parameters

IRLS score	*r*	*p*
Age (year)	− 0.075	0.531
Gender (male)	0.138	0.248
Duration of HD (months)	− 0.021	0.862
Body mass index (kg/m^2)	0.226	0.056
DM	− 0.090	0.453
HT	− 0.990	0.407
Cardiovascular disease	− 0.310	0.794
Polyneuropathy	0.121	0.312
FPG (mg/dL)	0.043	0.723
HbA1C	0.426	0.034
Predialysis urea (mg/dL)	− 0.082	0.500
Predialysis creatinine (mg/dL)	0.097	0.422
Hemoglobin (g/dL)	0.073	0.549
Uric acid (mmol/L)	0.166	0.169
Sodium (mmol/L)	− 0.059	0.629
Potassium (meq/L)	0.017	0.890
Calcium (mg/dL)	− 0.086	0.480
Phosphorus (mg/dL)	0.146	0.228
CaXP	0.147	0.224
intact PTH (pg/mL)	0.011	0.930
Albumin (g/L)	0.119	0.320
Iron (μg/dL)	0.218	0.070
TIBC (g/L)	0.001	0.991
TSAT (%)	0.189	0.125
Ferritin (ng/mL)	0.240	0.046
CRP (mg/dL)	− 0.114	0.352
IL-6 (pg/mL)	− 0.125	0.296
Hepcidin (ng/mL)	0.387	0.001

IRLS RLS Study group rating scale, *r* Spearman correlation coefficient, *p* *p* value, *HD* hemodialysis, *DM* diabetes mellitus, *HT* hypertension, *FPG* fasting plasma glucose, *HbA1C* hemoglobin A1C, *CaXP* calcium and serum phosphorus, *PTH* parathormone, *TIBC* total iron-binding capacity, *TSAT* transferrin saturation, *CRP* C-reactive protein, *IL-6* interleukin 6

evaluations of iron levels due to the high cost. The important roles of iron level, erythropoiesis, and inflammation have been demonstrated in the regulation of hepcidin production; however, there are many issues that are yet to be enlightened [17]. In inflammatory conditions, increases of such inflammatory cytokines as IL-6 and systemic iron accumulation lead to increased serum hepcidin levels, while iron losses decrease serum hepcidin levels [3, 17]. The level of circulating hepcidin increases noticeably as a result of increased inflammatory stimulation and decreased renal clearance in chronic HD patients [3, 17]. Serum hepcidin levels increase two- to four-fold in pre-dialysis patients and increase six- to nine-fold in patients undergoing HD [18]. There is a significant relation between hyperglycemia, hyperglycemia-induced oxidative stress, inflammation, and the development and progression of type 2 DM [19]. IL-6 has upregulated in type 2 DM and plays a role in the development of insulin resistance and also increases the expression of hepcidin [20]. Diseases with an ESDR etiology such as HT and DM, as well as increased comorbid disease load, contribute to increased inflammation in chronic HD patients [1]. In a study of diabetic CRF patients, increased hepcidin level has independently been associated with CRF progression [21].

Iron extraction from the cells to the circulation occurs through the mediation of ferroportin. Ferroportin levels decrease with the binding of hepcidin, and as a result, the iron levels in circulation drop. Increased hepcidin causes decreased iron levels and decreases the utility of iron by the brain and other organs [1]. Increased hepcidin has also been demonstrated to decrease iron release from astrocytes to CSF in a cell culture study in an in vivo iron deficiency model [22]. Plus, increased hepcidin decreases iron utility in the production of red blood cells, leading to the anemia of chronic disease [23].

Iron replacement is recommended in patients with CRF when TSAT and serum ferritin levels are lower than 20% and 100 ng/mL, respectively [24]. The iron absorption from the intestines and release from the reticuloendothelial system deteriorates due to the increased serum hepcidin levels in patients with ESRD. As such, oral iron for the treatment of anemia is useless, and thus, generally intravenous iron is adopted as a treatment [1].

Inflammation and related conditions are not dominant in the initiation phase of CRF, while the frequency of iron deficiency, peripheral neuropathy, inflammation, immune diseases, and RLS gradually increases as the disease advances [1]. Furthermore, serum ferritin and hepcidin levels are increased due to chronic inflammation in such patients. Iron deficiency in HD patients is considered to be linked to impaired iron utility, secondary to increased serum hepcidin levels due to systemic inflammation [3]. The improvement of iron utility following renal transplantation in such patients supports the thesis that inflammation is a mediator of RLS [1]. Although serum ferritin and TSAT levels have been reported to possibly be low in patients with primary RLS when compared with the healthy population, no significant difference has been found in the iron, ferritin, and TSAT levels between HD patient groups with and without RLS in the majority of studies performed [12, 14]. Serum ferritin levels were found to be low or high in a small number of studies of HD patients with RLS when compared with HD patients without RLS [25, 26].

The markers of inflammation and oxidative stress were also found to be high in a study, where ferritin was increased [26]. Hemoglobin and TSAT levels were similar in patients with and without RLS in the present study; however, serum ferritin and hepcidin levels were found to be significantly high in the group with RLS than in the group without RLS and were

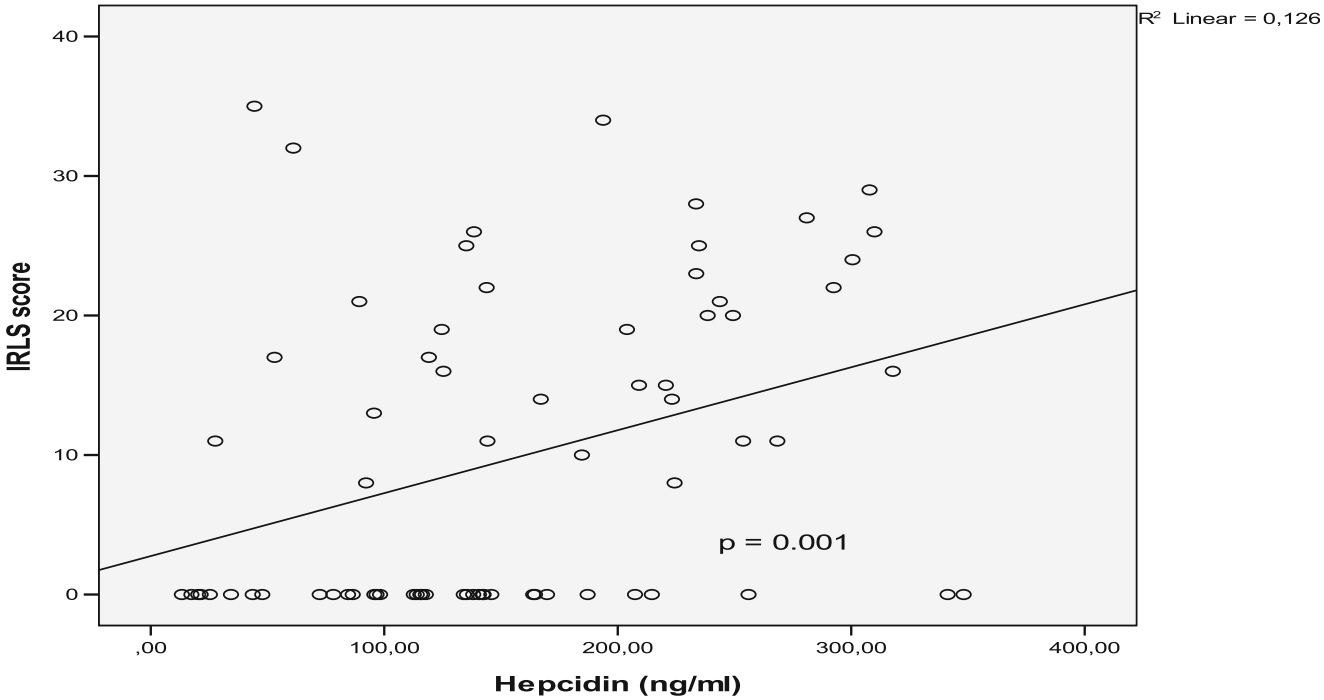

Fig. 1 Relation of hepcidin and IRLS score in HD patients with RLS

found to be positively correlated with the RLS severity. The multivariate linear regression analysis demonstrated that only hepcidin among these two parameters had an independent relation with the presence of RLS. Moreover, CRP and IL-6 levels, markers of inflammation, were evaluated, and no difference was found between patients with and without RLS. Additionally, no correlation between these parameters and the IRLS score was found. These findings suggest that inflammatory pathways may have a greater impact than anemia on the increased frequency and severity of RLS in chronic HD patients.

The relation between hepcidin and RLS has been investigated in a limited number of studies. In the first study in literature in which a limited number of cases were evaluated, pro-hepcidin levels were found to be increased in the neuromelanin cells in the brain parenchyma and substantia nigra while being decreased in CSF of the early phase primary RLS cases [5]. Serum hepcidin levels in patients with primary RLS (median: 13.95 ng/mL; range: 0.00–132.07) were found to be significantly high when compared with the control group (median: 2.55 ng/mL; range: 0.00–55.86), although no difference was noted in the ferritin levels in the first study evaluating RLS and serum hepcidin levels. Furthermore, a possible link was found between serum hepcidin level and the clinical severity of RLS [7].

Table 3 Linear regression analysis of hepcidin as a predictor of RLS

Variable	Beta	*p*	95% confidence interval	
			Lower	Upper
Hepcidin	0.482	0.035	0.000	0.006
HbA1C	0.418	0.035	0.012	0.293
Ferritin	− 0.234	0.284	− 0.001	0.000

RLS restless legs syndrome, *p p* value, *HbA1C* hemoglobin A1C

In another study, periodic leg movements during sleep and the relation with hepcidin levels were evaluated in patients with obstructive sleep apnea syndrome. Serum hepcidin levels and periodic leg movements in sleep were found to be increased in patients with obstructive sleep apnea when compared with patients with no obstructive sleep apnea, and the increase in serum hepcidin levels was demonstrated to be correlated with the increase in periodic leg movements. In addition, inflammation has been suggested to contribute to the increase in the serum hepcidin levels in such patients [27].

The present study for the first time evaluates the relation between RLS and hepcidin in chronic HD patients. The results point to an independent relation between RLS and hepcidin in chronic HD patients and indicate that a serum hepcidin level above 165 ng/mL may have a positive predictive value for the presence of RLS. Important biological mechanisms, including activation of hypoxic pathways, are manifested as a result of the effects of iron deficiency in RLS. The activation of hypoxia-inducible factor (HIF)-1α in the cerebral microvascular endothelial cells in patients with RLS increases the permeability of the blood-brain barrier, and hence, the regulation mechanisms of iron throughout the blood-brain barrier are affected as a result [11]. Increased inflammation has also been reported to contribute to the activation of HIF-1α [1].

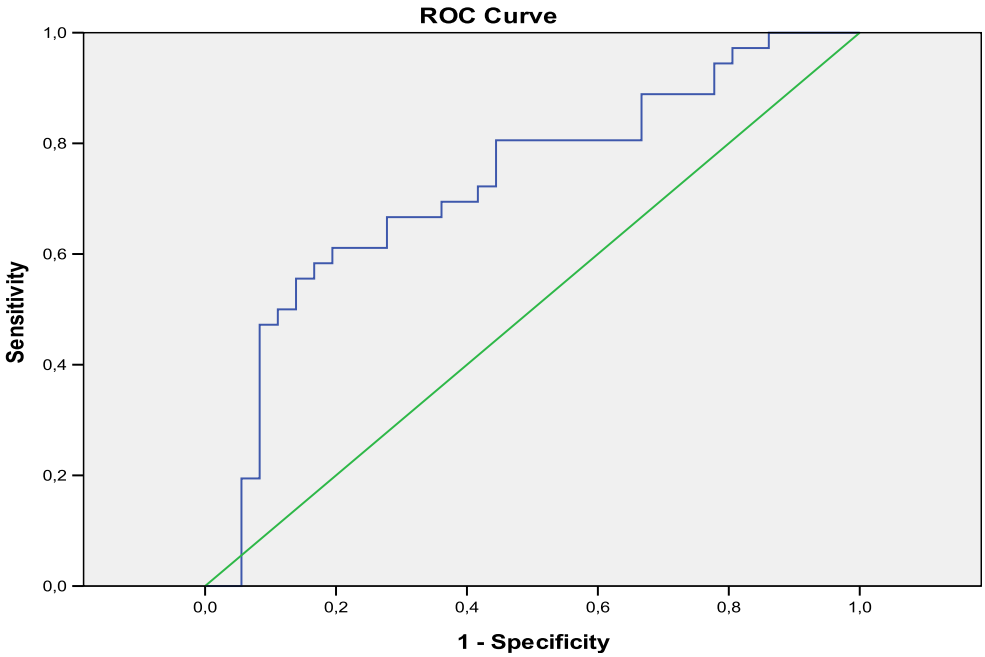

Fig. 2 ROC analysis of the relation of serum hepcidin and RLS

Similarly, HIF-2α activation has also been determined in the microvascular endothelial cells in patients with RLS [11].

The frequency and severity of RLS are increased in many diseases in which microvascular endothelial damage is present. The activation of the peripheral hypoxic pathways as a result of iron deficiency contributes to this increase [1, 11]. It has been reported that comorbidity of small fiber neuropathy, which is considered to play a role in the etiology of microvascular endothelial damage, may contribute to the emergence of clinical findings of RLS [4]. Mortalities associated with cardiovascular events are 10–20 times higher in chronic HD patients than in the general population [28]. The increased hepcidin-25 level in HD patients was demonstrated in a study to increase the frequency of fatal and non-fatal cardiovascular events, and an increase in cardiovascular events was associated with iron accumulation in the macrophages of the atherosclerotic plaques and oxidative stress [29]. Accompanying RLS in chronic HD patients was reported to increase mortality in a study, although this has not been supported in subsequent studies [25, 28]. No difference was found in the frequency of cardiovascular disease in patients with and without RLS in the present study. Our study is a cross-sectional one and patients were not followed up in terms of mortality.

Another factor that has been suggested to play a role in the pathophysiology of RLS in patients with CRF is calcium and phosphate imbalance. Dopamine mediates phosphate clearance in the kidneys, and diseases such as RLS, in which dopaminergic neuronal activity is impaired, are considered to accompany hyperphosphatemia [12]. Hyperphosphatemia accompanying HD has been demonstrated to increase the frequency of RLS in some studies, although there are other studies reporting no such relation [12, 13]. No difference was found in serum calcium, phosphate, or intact parathormone levels in chronic HD patients with and without RLS in this present study.

DM is among the first rank disorders in the etiology of ESRD in our country, as is the case in many countries. The prevalence of RLS is reported to be increased among patients with DM in studies carried out to date; however, it is undecided whether the DM itself or the development of polyneuropathy is the main factor [4]. RLS was found in 44.6% of all patients in a cross-sectional study comparing patients with type 2 diabetes mellitus with and without RLS. Among the patients included in the study with RLS, 44.4% were reported to require insulin to control their diabetes and to have a weak diabetic control [30]. In a study in our country, the incidence of RLS was reported to be high even after excluding polyneuropathy in patients with DM, and DM and duration of insulin use were demonstrated to be associated with RLS [31]. Although DM alone increases the risk of RLS, no increase in RLS has been identified in patients with DM and HD. In a large cross-sectional study of chronic HD patients, RLS was detected in almost one-fifth of patients, and no difference was found in the prevalence of DM in patients with and without RLS [32]. No difference was noted in the frequency of DM and polyneuropathy in patients with and without RLS in the present study, although HbA1C levels were found to be significantly high in the RLS group, and a significant correlation was identified between elevated HbA1C and IRLS scores.

Conclusions

The results of the present study show that a significant relation exists between increased serum hepcidin level and RLS in

chronic HD patients. A serum hepcidin level above 165 ng/mL may be a positive predictive factor for the presence of RLS, and uncontrolled diabetes may contribute to this relation. We suggest that the evaluation of serum hepcidin levels in chronic HD patients with complex iron metabolisms may contribute to a diagnosis of accompanying RLS. Future studies are required on this subject.

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Compliance with ethical standards

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Informed consent Consent form was filled out by all participants.

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