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Research Article

Clinical Usefulness of the serum-Soluble Transferrin Receptor in Iron Dysregulation Associated with long-Term Hemodialysis

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Abstract:

Background: Soluble transferrin receptor (sTfR) was reported to be a valuable diagnostic marker for iron dysregulation (ID). Hepcidin, which is a key regulator of iron metabolism, has been shown to have increased values in patients undergoing hemodialysis (HD). Either treatment with erythropoietin stimulating agent（ESA） or iron repletion therapy（IRT）had been reported to induce controversial effect on serum levels between sTfR and hepcidin. Thus, we measured serum levels both of sTfR and hepcidin in 97 HD patients and investigated the clinical value of these measures.

Methods: We used a commercial enzyme-linked immunosorbent assay kit to measure serum levels both of hepcidin and sTfR.

Results: Although serum sTfR levels did not clearly increase, both sTfR levels and its index value correlated with the saturation of transferrin, erythropoietin-stimulating agent dosage, and erythropoietin resistance index (ERI). In addition, a strong negative correlation was found for sTfR and log serum hepcidin values.

Conclusions: Serum sTfR levels and the sTfR index were acceptable markers for iron dysregulation (ID) and ERI even in patients undergoing HD whose serum levels of hepcidin increased.

Key words: hemodialysis; iron dysregulation; soluble transferrin receptor; hepcidin

Background

Iron dysregulation (ID) is one of most common complications associated with patients undergoing long-term hemodialysis (HD). More than half of HD patients need treatment with an erythropoietin-stimulating agent (ESA), which leads to a greater iron demand because of enhanced erythropoiesis. In fact, most HD patients treated with an ESA had several kinds of iron repletion therapy (IRT). Thus, monitoring and modulation of the body's iron status have become important routine works in HD. Iron metabolism in patients on maintenance HD is compromised by several factors including hepcidin. Hepcidin is generally acknowledged to be a key modulator in iron metabolism and reportedly increased in HD patients [1]. Hepcidin is mainly produced in hepatocytes and is known to degrade ferroportin on cell membranes, i.e. an exclusive exporter of intracellular iron. Although hepcidin was acknowledged to be a causative factor in ID associated with chronic inflammation [2, 3], its clinical significance has not been fully determined in the clinical setting described here, especially in patients undergoing ESA treatment and/or IRT.

In addition to hepcidin, a transferrin receptor (TfR) is a key regulator of iron, and its circulating levels have served as a marker for ID. A circulating TfR, i.e. soluble TfR (sTfR), is derived mainly from TfR1 expressed on cell membranes and is the main iron importer into cells, in contrast to ferroportin, which is an exporter. In addition, recent clinical studies indicated that the serum sTfR/log ferritin (Ft) ratio (sTfR index) was a useful index of ID [4–6]. However, the diagnostic significance of this index has not been fully studied in HD patients, whose serum levels of hepcidin are expected to be elevated.

In addition, both serum hepcidin and sTfR levels are likely to vary controversially according to the IRT and ESA treatment [8,15,17]. It thus seems important to understand the relationship between these two iron modulators in HD patients. In this study, we measured the serum levels of both hepcidin and sTfR in 97 HD patients, and we investigated their relationship with the patients' iron status. In addition, we discussed the

clinical usefulness of both serum sTfR levels and the sTfR index and the implications of hepcidin for these parameters.

Methods

Subjects

Ninety-seven patients, 52 male and 45 female, receiving maintenance HD and 16 healthy volunteers, 8 male and 8 female, were subjects in our study; all gave informed consent for participation. The mean ages (± standard error of the mean) of the patients and volunteers were 72.9 ± 10.5 (range 46–94) years old and 49.9 ± 8.3 (range 37–66) years old, respectively. Patients' primary diseases included chronic kidney disease (CKD) plus non-diabetes mellitus diseases in 57 patients, and plus diabetes mellitus in 40 patients. The mean duration of dialysis was 119.8 ± 106.6 months. Sixty-six patients were undergoing regular HD three times a week, and 31 patients were undergoing online hemodiafiltration (HDF) three times a week.

The treatment index *Kt/V* is used to assess dialysis dose, and its mean values, according to a single-pool model, were 1.30 ± 0.29 in patients receiving regular HD and 1.36 ± 0.23 in patients receiving HDF (p = 0.327) [7].

Patients with active sign of inflammation, bowel hemorrhage or liver damage and patients freated with HIF PH inhibitor were excluded from this study.

Measurement of study parameters

At the start of HD on the first day of the week, we obtained blood samples via an arterial line. All parameters shown in Table 1 were measured by Falco Laboratory (Kyoto, Japan). Serum levels of bioactive hepcidin were measured at Kurume University by using commercial kits (DRG Instruments GmbH, Marburg, Germany), and serum levels of sTfR were measured similarly by using commercial kits (Abcam plc, Cambridge, UK).

ESA treatments and IRT

Three ESAs—epoetin beta, darbepoetin alfa, and epoetin beta pegol—were given to 82 patients. Each ESA dosage was converted to an epoetin beta unit according to previous reports, and the mean dosage was 74.86 ± 49.65 IU/kg body wt/W [8]. ERI values were calculated by using a formula as a weekly ESA dose/kg body wt/Hb [9]. The mean ERI value was 7.30 ± 5.34 (range 0.63–7.22). Forty-nine patients received IRT by means of saccharated ferric oxide (Fesin) (Nichi-Iko Pharmaceutical Co. Ltd., Tokyo), at mean dose of 1.678 ± 0.951 mg/kg/M.

Statistical analysis

Data were analyzed by student's t test or Peason's method using JMP version 9.0.0 (University of California, Merced, CA). Data are presented here as the mean ± standard error of the mean. A p value of less than 0.05 was considered to be statistically significant.

Results

Basic laboratory data

Table 1 presents basic laboratory data of the study participants. The mean saturation of transferrin (TSAT) value was 29.4 ± 10.5% (13–69%), and the mean serum Ft concentration was 169.1 ± 121.7 ng/ml (17–602 ng/ml). No patient with C-reactive protein levels higher than 0.5 mg/dl was included.

Parameter	ESRD patients (n = 97)	Healthy volunteers (n = 16)
Albumin (g/dl)	3.63 ± 0.32	4.46 ± 0.21
Creatinine (mg/dl)	10.3 ± 2.69	0.76 ± 0.16
C-reactive protein (mg/dl)	0.20 ± 0.30	0.04 ± 0.08
Iron (μg/dl)	70.9 ± 26.3	104.9 ± 27.0
Ferritin (ng/dl)	169.2 ± 122.3	73.4 ± 56.3
TSAT (%)	29.4 ± 10.5	30.4 ± 11.3
Hepcidin (ng/ml)	29.7 ± 26.0	9.8 ±5.8
sTfR (ng/ml)	1387.4 ± 599.3	1124.2 ± 370.9

Abbreviation: ESRD, end-stage renal disease.

Table 1: Basic laboratory data of study participants

Serum levels of hepcidin

Although 22 patients had low levels of serum hepcidin (<10 ng/ml), levels of hepcidin were as much as 3-fold higher than controls, on average—29.7 ± 26.0 ng/ml (2.3–110.8 ng/ml) vs. 9.8 ± 5.8 ng/ml (4.6–25.1 ng/ml) (p = 0.003)—and these values showed a strong positive correlation with serum Ft values (r = 0.531, p < 0.001) (data not shown). No significant difference was found between the patient subgroup undergoing regular HD and that undergoing HDF (30.4 ± 26.2 vs. 28.3 ± 26.0 ng/ml, p = 0.71). Serum hepcidin levels were, albeit not significant, higher in patients having IRT compared with levels in patients not having IRT (33.4 ± 25.7 vs. 25.9 ± 26.1 ng/ml, p = 0.157). Serum hepcidin levels were 29.3 ± 25.0 ng/ml in patients undergoing ESA therapy and 32.3 ± 32.0 ng/ml in those not undergoing ESA therapy (p = 0.69). No correlation could be confirmed between serum hepcidin levels and either ESA dosage or iron dosage (data not shown). Also, no correlation was found between serum hepcidin levels and TSAT values or ERI values (data not shown).

Serum levels of sTfR

Serum sTfR levels were moderately higher in HD patients than in controls (1387.4 ± 599.3 vs. 1124.2 ± 370.9 ng/ml, p = 0.0919). No significant difference was seen in serum sTfR levels between the patient subgroup undergoing regular HD and that undergoing HDF (1428.4 ± 585.5 vs. 1300.0 ± 628.5 ng/ml, p = 0.328). In addition, no significant difference in serum sTfR levels was found for patient subgroups with and without IRT or with and without ESA therapy (1317.4 ± 519.8 ng/ml vs. 1458.9 ± 668.8 ng/ml; 1384.9 ± 547.4 ng/ml vs. 1401 ± 853.6 ng/ml, respectively). However, a good correlation existed between serum sTfR levels and ESA dosage (r = 0.348, p = 0.00136) (Figure. 1a).

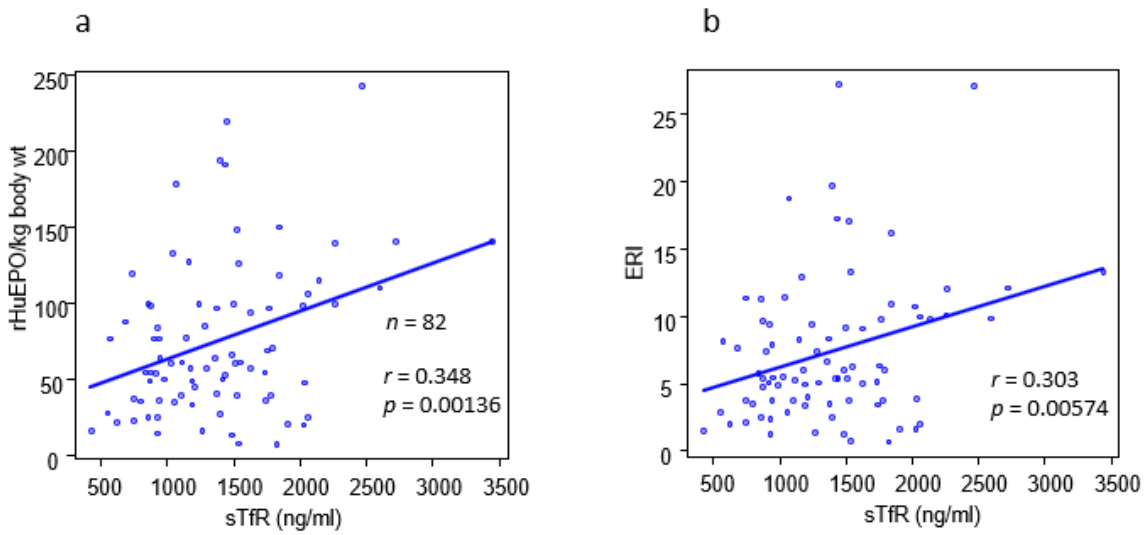

Figure 1: Correlation between serum sTfR levels and ESA dose (a) and ERI (b). **Abbreviation:** rHuEPO, recombinant human erythropoietin.

Similarly, a good correlation was found between serum sTfR levels and ERI values (Figure. 1b). In addition, serum sTfR levels were inversely correlated with TSAT values (r = -0.27, p = 0.00743) (Figure. 2).

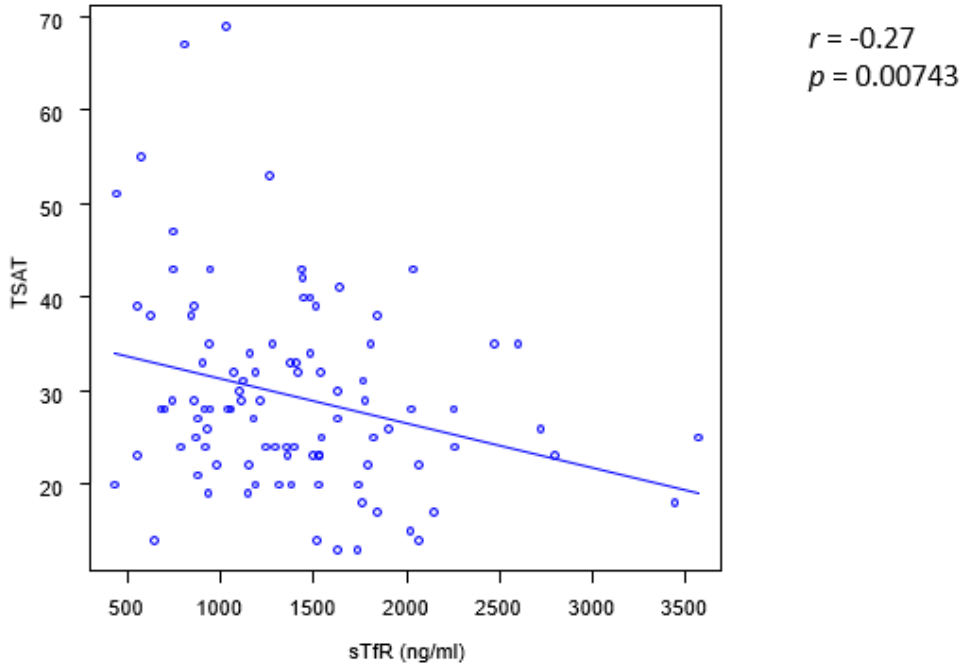

Figure 2: Correlation between TSAT and serum sTfR values.

Serum sTfR index

Serum sTfR index values in HD patients were not significantly different from those in controls (304.8 ± 165 vs. 319.0 ± 153.9, p = 0.746). However, this index correlated strongly with TSAT values and with either ESA dosage or ERI (Figure. 3 and 4). No correlation was found between the sTfR index and the iron dosage (p = 0.877) (data not shown).

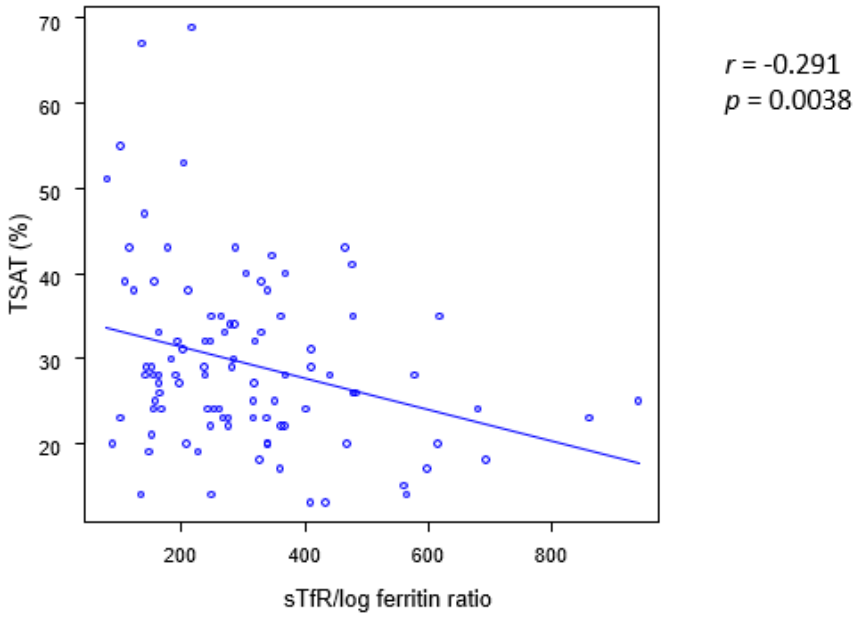

Figure 3: Correlation between TSAT and sTfR index.

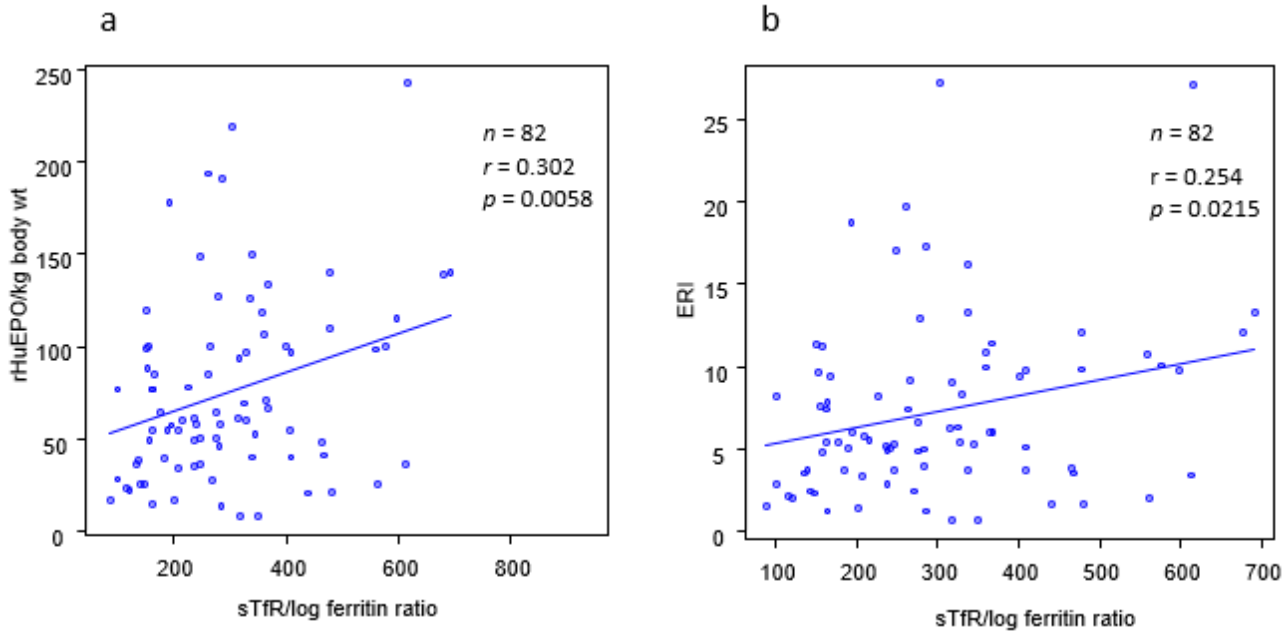

Figure 4: Correlation between sTfR index and ESA dose (a) and ERI (b).

Correlation among parameters related to hepcidin and sTfR

As expected from the converse effects of ESA therapy on serum levels of hepcidin and sTfR, a strong inverse correlation was found between hepcidin and both serum sTfR levels and the sTfR index (Figure. 5). In particular, a correlation between the log serum hepcidin values and the sTfR index was confirmed with extremely high significance ($r = 0.523$, $p < 0.0001$).

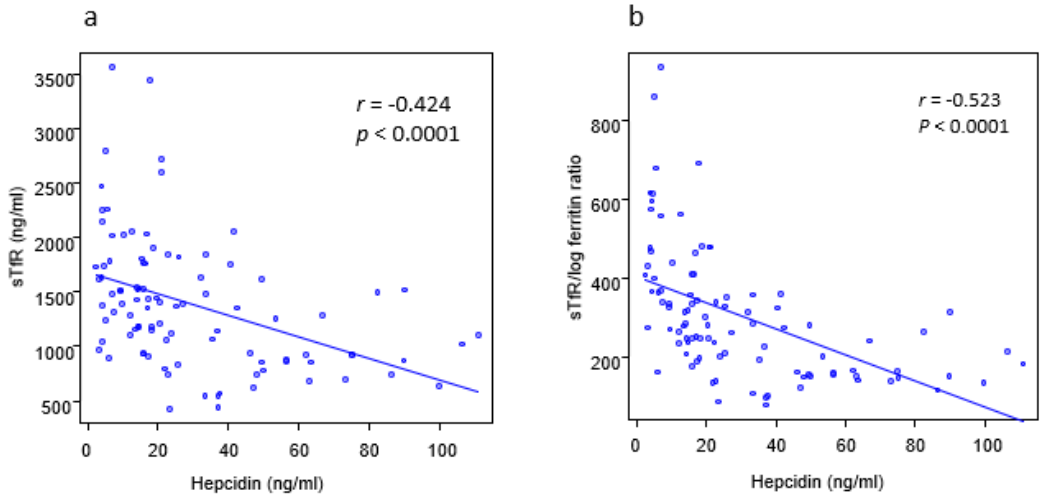

Figure 5: Correlation between serum hepcidin levels and serum sTfR values (a) and the sTfR index (b).

Discussion

ID has been well-known as a common complication associated with CKD [10]. In addition, current ESA treatments in CKD patients increase the iron demand to respond to stimulated erythropoiesis. Consequently, most HD patients undergoing ESA treatment must have several types of IRT.

Thus, to minimize the generation of radical oxygen species caused by ferrotoxic action, iron dosages have been monitored according to the percent TSAT, with those values kept at roughly 20% or more. However, TSAT values may still be problematic as an index of ID in this clinical setting [11].

ID in HD patients is multifactorial and mostly complicated by chronic inflammation related to oxidative stress associated with long-term HD, which is believed to stimulate production of hepcidin via interleukin-6 in the liver [12, 13]. Hepcidin was believed to be a key factor in iron metabolism and to impair iron trafficking including mobilization from tissues into systemic circulation. Hepcidin was reportedly thought to be a causative factor of functional iron deficiency in HD patients [14].

Hepcidin production was also stimulated directly by iron loading [15] and conversely was suppressed directly by erythroferrone during ESA treatment, as reported previously [8]. In this study, we could not confirm significant differences in serum hepcidin levels among patient subgroups with vs. without IRT or ESA treatment, nor could we correlate serum hepcidin levels with TSAT, iron dosage, and ESA dosage. However, we believe that those treatments compromised a direct correlation among them and that hepcidin is a major causative factor in ID in HD patients as well as in other inflammatory diseases. In fact, a logarithmic transformed serum hepcidin level correlated strongly with the sTfR index, which is known as a marker for ID (Figure. 5b).

Serum sTfR levels reportedly increased exclusively in functional iron deficiency [16]. In addition, serum sTfR levels increased during ESA treatment [17]. However, in this study the sTfR levels did not increase as clearly as in reports of other anemic diseases associated with chronic inflammatory conditions [18]. Nevertheless, a significant negative correlation of TSAT was confirmed with either sTfR or the sTfR index, which suggests that those parameters are useful as markers for ID as reported previously [19, 20]. In particular, as reported earlier, the sTfR index seemed superior to serum sTfR levels, even in HD patients undergoing IRT, because of higher significance in Figure. 3, which indicated that the index of 800 corresponded to 20% of TSAT [21, 22].

Serum sTfR levels depend on the extent of expression of TfR1 on cell membranes, which is regulated by a hypoxia-inducible factor and an iron regulatory protein (IRP)/iron-responsive element (IRE) system. The IRP/IRE system function depends on cellular free iron levels [23, 24].

Hepcidin was reportedly excreted in urine [25], which means that serum hepcidin levels before HD were elevated in all patients with end-stage kidney disease. Thus, for iron loading when high hepcidin levels exist, such as in our patients, intracellular iron levels may increase via TfR and inactivate the IRP/IRE system, which would turn off translation of sTfR mRNA and turn on translation of Ft mRNA. As a consequence, an increase in sTfR associated with iron deficiency was thought to be likely offset by inactivated IRP during high levels of hepcidin.

In addition, TfR expression is most abundant in erythroblasts and has reportedly increased along with enhanced erythropoiesis after ESA treatment. We also found a correlation with ESA doses in this study, which suggests that both serum sTfR levels and the sTfR index are possible markers for ERI despite a coexisting increase in hepcidin [26]. We did confirm a good correlation between ERI and both serum sTfR levels and the sTfR index. ERI is determined mostly by a weekly dosage of ESA [9]so a correlation with the ESA dosage is likely to result in a correlation with ERI as well.

Belo et al. also recently demonstrated that serum sTfR levels were a suitable indicator of ERI in HD patients [17]. Our study thus suggested that the sTfR index may be a valuable marker for ERI as well as serum sTfR values (Figure. 4b). In addition, the sTfR index is expected to serve as an aid to monitor iron supplementation because of the lack of differences in basal levels compared with controls (Figure. 4b).

Finally, a negative correlation between serum levels of hepcidin and sTfR might be due to antagonistic effect on both IRT and ESA therapy on their serum levels rather than due to direct interaction with each other.

Conclusion

Our study showed that both serum sTfR levels and the sTfR index served as indicators of ID and ERI, but the clinical utility of these indicators was compromised by hepcidin, which was elevated by renal failure, subclinical inflammation associated with long-term HD, and iron loading. Although we could not provide direct proof of the involvement of hepcidin with ID, compared with TSAT, a logarithmic value of serum hepcidin levels correlated strongly with the sTfR index, which indicated strong implication of hepcidin with ID in HD patients. We therefore should not ignore the implications of hepcidin. Our study thus indicated a clinical value of sTfR in the diagnosis of ID even with high levels of serum hepcidin.

This study had several limitations. First, it was a cross-sectional study of 96 patients who were older than the control subjects. Next, our patients underwent two types of HD treatment, i.e. regular and online HDF. Last, blood was sampled only once at different times, including 7:30 AM, 12:30 PM, and 16:30 PM.

Declarations

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee of Suiyukai (protocol code 014, March 15th, 2019).

Consent for publication

Not applicable

Availability of data and materials

All data generated or analysed during this study are included in this published article [and its supplementary information files].

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The authors declare that they have no other relevant financial interests.

Competing interests

The authors declare no competing interests.

Authors' contributions

Research idea and study design: **YM, YK;** data acquisition: YM, **YH;** data analysis/interpretation: YM, **YK;** statistical analysis: **YH;** supervision or mentorship: YM. Each author contributed important intellectual content during manuscript drafting or revision and agrees to be personally accountable for the individual's own contributions and to ensure that questions pertaining to the accuracy or integrity of any portion of the work, even one in which the author was not directly involved, are appropriately investigated and resolved, including with documentation in the literature if appropriate.

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