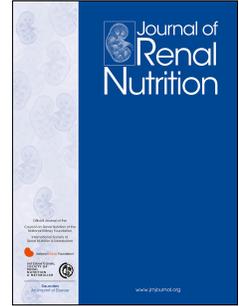


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Relation of Liver Siderosis to Liver Fibrosis in Hemodialysis Patients with Severe Hyperferritinemia Secondary to High Doses of Intravenous Iron Supplementation

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Relation of Liver Siderosis to Liver Fibrosis in Hemodialysis Patients with Severe Hyperferritinemia Secondary to High Doses of Intravenous Iron Supplementation

Abstract

Background

Aggressive iron substitution in hemodialysis (HD) patients leads to iron overload. The association between liver siderosis and fibrosis is still debatable. We studied the association of liver siderosis with liver fibrosis in HD patients. Furthermore, we studied the performance of liver stiffness measurements in identifying advanced liver fibrosis. We investigated the performance of biochemical indicators of iron status in identifying advanced liver fibrosis.

Patients and methods

Fifty-five HD patients (average hemodialysis duration 6 ± 2 years) with hyperferritinemia secondary to intravenous iron supplementation (weekly iron dose 252.7 ± 63 mg; median blood transfusions 3 [2-5]) were recruited. The liver fibrosis grade was determined with FibroScan, APRI, and Fib-4 index. Liver iron concentration (LIC) was estimated with MRI. Iron parameters and liver function biochemical indicators were also assessed.

Results

The median serum ferritin and TSAT were $3531 \mu\text{g/l}$ and 77%, respectively. 34.5%, 20%, and 45.5% of the patients showed mild, moderate, or severe liver siderosis, respectively. All patients with severe liver siderosis showed advanced liver fibrosis. Patients with severe liver siderosis and advanced liver stiffness showed higher serum iron, TSAT, AST, ALT, serum bilirubin, APRI,

and Fib-4 index scores than those with mild liver siderosis. Serum iron and TSAT showed good utility in identifying advanced liver fibrosis determined with FibroScan, APRI, and Fib-4 index. Liver stiffness exhibited good utility in identifying advanced liver fibrosis diagnosed with APRI and Fib-4 index.

Conclusions

High weekly intravenous iron dose associated with severe hyperferritinemia, high serum iron, and TSAT might lead to severe liver siderosis and concomitant liver fibrosis in HD patients. Serum iron and TSAT, Fibroscan, Fib-4 and APRI scores might offer non-invasive tools for identifying advanced liver fibrosis in those patients.

Keywords

Siderosis, liver fibrosis, APRI, Fib-4, FibroScan, LIC

Introduction

Anemia is one of the common complications of chronic kidney disease (CKD). Iron deficiency is a major cause of anemia in this patient population. Iron substitution plays a pivotal role in the management of renal anemia in CKD and hemodialysis (HD) patients. Policies of administration of high iron doses (300-400 mg/month) have been reported by many North American and European dialysis centers to reduce the dose of the expensive erythropoietin stimulating agents (ESA) ¹. Long-term iron administration and overtreatment of iron deficiency might result, however, in iron overload. Safety of long-term iron administration in dialysis patients has been controversially discussed.

MRI is the tool of choice for quantification of hepatic and extrahepatic iron concentration in iron overload disorders ^{2,3}. Increased prevalence of liver siderosis in HD patients was reported irrespective of adjustment of iron dose to the recommended target serum ferritin levels ^{4,5}. Despite these findings, whether liver fibrosis or cirrhosis represents the endpoint of liver siderosis in dialysis patients remains debatable in the view of normal liver enzymes ⁶⁻⁸ and the rare association of severe liver siderosis with post-mortem biopsy-proven liver fibrosis in the pre-ESA era ^{6,8,9}.

Liver biopsy is the gold standard for diagnosis of liver fibrosis. Being invasive and unsuitable for follow-ups, alternative tools have been investigated. A meta-analysis showed that transient elastography (FibroScan) and APRI (aspartate aminotransferase [AST]-to-platelet ratio index) score provided a sensitivity of 83.4% and 66.5% and a specificity of 92.4% and 71.7%, respectively, in identifying stage IV biopsy-proven liver fibrosis (cirrhosis) ¹⁰. A recent study on

hemochromatosis patients with variants in the homeostatic iron regulator gene reported that APRI cut-off of 0.44 showed 79.4% sensitivity and 89% specificity for detection of biopsy-proven advanced liver fibrosis stages (F3 and F4), whereas fibrosis-4 (Fib-4) index cut-off of 1.1 showed 80% sensitivity and 80.3% specificity for detection of biopsy-proven advanced liver fibrosis stages (F3 and F4) ¹¹.

In the current study we aimed to provide evidence on the association of severe liver siderosis (estimated with MRI) with advanced liver fibrosis with the aid of FibroScan, APRI, Fib-4 index, and other biochemical indicators of liver fibrosis in hemodialysis patients. Secondly, we investigated the performance of liver stiffness measured with FibroScan in predicting advanced liver fibrosis estimated with the APRI and Fib-4 index scores. Lastly, we investigated whether commonly used iron parameters for diagnosis of iron overload (serum iron, serum ferritin, TSAT, and total iron binding capacity [TIBC]) might act as a valid tool for predicting the degree of liver fibrosis in HD patients.

Methods

Study design and participants

The current cross-sectional study was conducted in the Nephrology Unit of the Internal Medicine Department between May 2019 and May 2020. Fifty-five patients with end-stage kidney disease (ESKD) on regular hemodialysis for at least two years were enrolled in the study. Inclusion criteria also included a TSAT > 20% and a serum ferritin > 500 µg/dl. Patients with one or more of the following conditions were excluded; non-dialysis-dependent chronic kidney disease

patients (NDD-CKD), hepato-renal syndrome as the cause of ESKD, other causes of hemochromatosis such as hereditary type, hepatitis B or C virus infection, or human immunodeficiency virus infection. Moreover, patients with additional causes of liver disease, including non-alcoholic fatty liver disease, primary sclerosing cholangitis, primary biliary cholangitis, etc. were excluded. Other exclusion criteria included active malignancy, heart failure, use of immunosuppressive drugs, previous liver transplantation, and patients below 18 years of age.

Procedures and measurements

All recruited patients underwent the following investigations:

- Measurement of iron parameters, including serum iron, total iron-binding capacity (TIBC), and transferrin saturation (TSAT), and serum ferritin.
- Estimation of the biochemical indicators of liver function, including platelets, AST, ALT, serum bilirubin, serum total protein, and serum albumin.
- Calculation of non-invasive panel scores for liver fibrosis, including the aspartate aminotransferase-to-platelet ratio index (APRI) and the fibrosis-4 Index (Fib-4) as follows:

$$\text{APRI} = \frac{\text{AST level (U/L)} / \text{upper normal limit of AST (U/L)} \times 100}{\text{Platelets (109/L)}}$$

$$\text{Fib} - 4 = \frac{\text{Age (years)} \times \text{AST (U/L)}}{\text{Platelets (109/L)} \times \sqrt{\text{AST (U/L)}}}$$

- Ultrasound liver assessment by a single experienced operator. Patients were classified as either showing signs of liver cirrhosis or not.

- Liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE): liver stiffness was assessed using FibroScan 502 Touch devices equipped with both M and XL probes (Echosens, Paris, France) as described by Sasso et al ¹². LSM was determined as the median of 10 measurements ¹³. According to the measured liver stiffness value, the degree of liver stiffness (measured in kilopascals [kPa]) was classified into mild (LSM < 9.5 kPa) or advanced liver fibrosis (LSM ≥ 9.5 kPa). This cut-off was derived from the study of Castera et al ¹⁴. LSM < 9.5 kPa corresponded to F0-F1 and F2 stages, whereas LSM ≥ 9.5 kPa corresponded to F3-F4 stages in the METAVIR scoring system for liver biopsies ¹⁴.
- Liver iron measurement by Magnetic Resonance Imaging (MRI): MRI measurements were performed with a Philips Achieva 1.5T MRI machine (Philips, Netherlands) by the same experienced operator who was blinded to patients' clinical and laboratory data. Iron therapy was postponed so that at least one week without iron administration elapsed before conducting MRI. Three MRI methods were applied to estimate LIC qualitatively; signal intensity ratio (SIR) according to Rennes University ¹⁵, R2* relaxometry, and R2* relaxometry with multi-p recommendations ¹⁶. The degree of liver iron overload was classified as normal load (T2* > 11.4 ms corresponding to LIC <2 mg/g dry liver weight), mild (T2* 3.8-11.4 ms corresponding to LIC 2 - 7 mg/g dry liver weight), moderate (T2* 1.8-3.8 ms corresponding to LIC 7 - 15 mg/g dry liver weight), and severe iron overload (T2* ≤ 2 ms corresponding to > 15 mg/g dry liver weight).
- Quantification of serum type IV collagen using commercially available ELISA kits according to manufacturer's recommendations (Echelon Biosciences Inc., Salt Lake City, Utah, USA).

Blood samples for evaluation of iron parameters, complete blood picture, liver function, and type IV collagen were obtained maximally 7 days prior to conduction of MRI and FibroScan.

The current study was conducted in accordance with the Code of Good Practice and the guidelines of the Declaration of Helsinki, 7th revision, 2013, and after being approved by the Medical Ethics Committee of the Faculty of Medicine with the IRB number 17200610. A written informed consent was obtained from all participants before being enrolled in the study.

Statistical analysis

Data were presented either as mean \pm standard deviation (SD), or as median and interquartile range (IQR). Mann-Whitney or Kruskal-Wallis test with Dunn's multiple comparisons test was performed to compare continuous variables. Correlations between continuous variables were conducted with Spearman rank correlation test. Categorical variables were compared with chi-square test. Univariable and multivariable logistic regression analyses with backward elimination method were conducted. Independent variables with significant associations in the univariable analysis ($p < 0.1$) were further included in the multivariable regression analysis.

Receiver operating characteristic (ROC) curve analyses were conducted. Optimal cut-off values were selected according to Youden's index¹⁷. The statistical analysis was performed with SPSS Statistics 26 (IBM, Ehningen, Germany). p -values < 0.05 were considered significant.

Results

Participants

A total of 55 patients were enrolled in the study. Only 27 % of patients had diabetes mellitus, whereas about 62% suffered from arterial hypertension. The hemodialysis regimen consisted of 3 dialysis sessions per week, each lasted for at least 4 hours. The mean dialysis duration was 6 ± 2 years. The mean weekly dose of intravenous supplemented iron was 252.7 ± 63 mg, whereas the median total number of blood transfusions since dialysis begin was 3 times (IQR [2-5]). Patients received blood transfusions whenever their hemoglobin level was below 7 g/dl. Initial dose of erythropoietin alpha was 50-100 units /kg administered 3 times/week. The median dose of intravenously administered erythropoietin alpha was 6000 IU (4000-8000) in the mild liver fibrosis group and 10000 IU (8000-12000) in the advanced liver fibrosis group.

The baseline characteristics and demographic data are shown in **Table 1**.

LIC in HD patients in relation to iron parameters

According to the degree of LIC, the study patients were classified into mild (34.5%), moderate (20%), or severe liver siderosis groups (45.5%). Moreover, we compared iron parameters (serum iron, serum ferritin, TSAT, and TIBC) among the 3 groups of liver siderosis. We found a sequential increase in serum iron and serum ferritin from the mild through the severe liver siderosis groups. Serum iron and TSAT differed significantly among the 3 groups ($p = 0.001$; $p = 0.026$, respectively). Post-hoc comparisons demonstrated that the serum iron and TSAT were significantly higher in the severe than the mild liver siderosis group ($p = 0.001$; $p = 0.029$, respectively). No significant difference could be detected either between the mild and moderate or between the moderate and severe groups. Neither serum ferritin nor TIBC differed significantly among the 3 groups ($p = 0.222$; $p = 0.814$, respectively) (**Table 2**).

Biochemical indicators of liver function in relation to the LIC

ALT, AST, ALP, serum bilirubin, serum total protein, serum albumin, APRI, and Fib-4 index scores were classified according to LIC estimated by MRI. ALT and serum bilirubin were significantly higher in the severe liver siderosis as compared to the mild liver siderosis group ($p < 0.001$, $p < 0.001$). ALT was significantly higher in the severe liver siderosis group compared with the low and moderate siderosis groups ($p < 0.001$, $p = 0.002$, respectively). Both serum total protein and serum albumin were significantly lower in the severe than in the mild and moderate liver siderosis groups ($p = 0.019$, $p = 0.006$; $p = 0.003$, $p = 0.043$, respectively). Although ALP was comparable among the three liver siderosis groups ($p = 0.05$), all groups showed higher than average ALP levels. This might be in part attributed to an increase in bone alkaline phosphatase (BALP). Unfortunately, BALP was not estimated in the current study. Patients with severe liver siderosis showed significantly higher APRI and Fib-4 index scores than those with mild or moderate liver siderosis (APRI: $p < 0.001$, $p = 0.007$, respectively; Fib-4: $p < 0.001$, $p = 0.034$, respectively). We found no difference in the serum collagen IV levels among the three liver siderosis groups (**Table 2**).

Liver stiffness estimated with FibroScan in HD patients

The median liver stiffness value was 8.5 kpa. Hemodialysis patients were divided into two groups according to their LSM; patients with values ≤ 9.5 kilopascal (kpa) were considered to have mild liver fibrosis ($n = 30$; 54.5 %), whereas patients with LSM values > 9.5 kPa were considered to have advanced liver fibrosis ($n = 25$; 45.5%). Both groups showed no significant difference regarding baseline characteristics. The advanced liver fibrosis patients received significantly more total blood transfusions and weekly erythropoietin-alpha dose than those with mild liver fibrosis ($p < 0.001$, $p < 0.001$, respectively). Moreover, the advanced liver fibrosis group showed higher ALT ($p < 0.001$), AST ($p < 0.001$), ALP ($p = 0.018$), total bilirubin ($p < 0.001$), whereas it

exhibited lower total serum proteins, serum albumin, and platelet concentration ($p < 0.001$; $p = 0.001$; $p < 0.001$, respectively). In addition, the advanced liver fibrosis group showed higher serum iron and TSAT than the mild liver fibrosis group ($p < 0.001$; $p = 0.008$, respectively). Neither the serum ferritin nor the TIBC differed significantly between the two groups ($p = 0.083$; $p = 0.526$, respectively). We found no significant difference in serum type IV collagen between the mild and advanced liver fibrosis groups (**Table 1**).

Relation between liver fibrosis severity (assessed with FibroScan) and LIC (assessed with MRI) in HD patients

We investigated the relation between the grade of liver siderosis (assessed with MRI) and the severity of liver fibrosis (estimated with FibroScan). A significant association could be detected (chi-square test, $p < 0.001$). All patients with severe liver siderosis showed advanced liver fibrosis ($n = 25$), whereas all patients with mild ($n = 19$) or moderate liver siderosis ($n = 11$) exhibited mild liver fibrosis.

Association of iron parameters with the grade of liver fibrosis estimated with FibroScan, APRI, or Fib-4 index

Univariable and multivariable logistic regression analyses including serum collagen IV, serum iron, serum ferritin, TSAT, and TIBC as potential predictors and liver stiffness grade as a binary outcome (mild vs. advanced liver stiffness) showed that only serum iron was independently associated with the liver fibrosis grade (odds ratio [OR] 1.027, 95% confidence interval [CI] 1.009 – 1.046, $p = 0.003$) (**S1 Table**). The liver siderosis grade was not included as a predictor in the logistic regression because of complete separation (perfect prediction). In the multivariable logistic regression with dichotomized Fib-4 and APRI scores as outcomes, only serum iron and

mild liver siderosis (in reference to severe liver siderosis) were independently associated with the Fib-4 and APRI scores, respectively (serum iron in reference to Fib-4: odds ratio [OR] 1.020, 95% confidence interval [CI] 1.004 – 1.036, $p = 0.015$; mild liver siderosis in reference to APRI score: odds ratio [OR] 0.113, 95% confidence interval [CI] 0.023 – 0.566, $p = 0.008$) (**S2 Table**).

Reliability of iron parameters in discriminating advanced liver fibrosis in HD patients

To assess the validity of serum iron, serum ferritin, TSAT, and TIBC in discriminating advanced liver stiffness or fibrosis determined with FibroScan, APRI, or Fib-4 scores, we conducted a ROC analysis. When the liver fibrosis grade estimated with FibroScan was used as the reference standard, the serum iron provided the highest AUC (0.804, $p < 0.001$; cut-off value 153 $\mu\text{g/dl}$; sensitivity 87.5 %; specificity 58.6 %), followed by TSAT (AUC = 0.710, $p = 0.009$; cut-off value 81%; sensitivity 67%; specificity 76%). Both serum ferritin and TIBC were not valid to detect advanced liver stiffness (AUC = 0.643, $p = 0.075$; AUC = 0.580, $p = 0.321$) (**Fig.1**). When Fib-4 score was dichotomized (≤ 1.1 vs. > 1.1) and used as the reference standard, the serum iron provided the highest AUC (0.778, $p = 0.001$; cut-off value 153 $\mu\text{g/dl}$; sensitivity 80%, specificity 72%), followed by TSAT (AUC = 0.737, $p = 0.005$; cut-off value 76.5%; sensitivity 69%; specificity 78%) and serum ferritin (AUC = 0.689, $p = 0.025$; cut-off value 1575 $\mu\text{g/l}$; sensitivity 97%, specificity 56%) (**Fig.2A**). Similarly, APRI score was dichotomized (≤ 0.44 vs. > 0.44) and used as the reference standard. Serum iron showed the highest AUC (0.777, $p = 0.001$; cut-off value 176.5 $\mu\text{g/dl}$; sensitivity 61%, specificity 82%), followed by TSAT (AUC = 0.725, $p = 0.006$; cut-off value 76.5%; sensitivity 71%, specificity 73%) and serum ferritin (AUC = 0.717, $p = 0.008$; cut-off value 1575 $\mu\text{g/l}$; sensitivity 97%; specificity 45%) (**Fig.2B**).

Performance of FibroScan score in detecting advanced liver fibrosis diagnosed with APRI and Fib-4 scores

32 of 55 patients (58.2%) showed APRI scores > 0.44 , which was shown to be consistent with advanced liver fibrosis¹¹, whereas 23 patients (41.8%) showed APRI scores ≤ 0.44 (no liver fibrosis). In the ROC analysis, LSM as a predictor of APRI score showed an AUC of 0.834 ($p < 0.001$; cut-off value 8.9 kPa; sensitivity 78%; specificity 99.95%) (**Fig. 3**). Those patients, classified as having advanced liver fibrosis using APRI scores > 0.44 , showed significantly higher TSAT and worse AST, ALT, bilirubin, serum total protein and serum albumin compared with those with APRI ≤ 0.44 . No significant difference in serum iron, serum ferritin or serum collagen IV could be detected between both groups ($P = NS$) (**Table 3**).

36 of 55 patients (65.5%) showed Fib-4 scores > 1.1 , which was shown to be consistent with advanced liver fibrosis¹¹, whereas 19 patients (34.5%) showed Fib-4 scores ≤ 1.1 (no liver fibrosis). In the ROC analysis, FibroScan scores as a predictor of Fib-4 score showed an AUC of 0.776 ($p = 0.001$; cut-off value 8.65 kPa; sensitivity 69%; specificity 89%) (**Supplementary Fig.1**). Those patients, classified as having advanced liver fibrosis using Fib-4 scores > 1.1 , showed significantly higher serum iron and TSAT, and worse AST, ALT, serum total protein and serum albumin compared with those with Fib-4 scores ≤ 1.1 kPa (**Table 3**). Taken together, LSM demonstrates a good utility for detection of advanced liver cirrhosis in HD patients.

Discussion

Iron substitution plays a pivotal role in the management of renal anemia in CKD patients.

According to the KDIGO-2012 guidelines for management of anemia with CKD, iron supplementation should be considered in anemic dialysis patients when TSAT $\leq 30\%$ and serum ferritin ≤ 500 ng/ml¹⁸. Recently, a randomized controlled trial showed that proactive intravenous iron administration in dialysis patients if TSAT $\leq 40\%$ or serum ferritin ≤ 700 $\mu\text{g/l}$ was

associated with lower frequency of the primary composite endpoint (myocardial infarction, cerebrovascular stroke, hospitalization for heart failure, or death) compared with the reactive low-dose iron administration. The association of liver siderosis with liver fibrosis in CKD and dialysis patients has been controversially discussed in the literature in the view of normal liver enzymes⁶⁻⁸ and the rare association of severe liver siderosis with post-mortem biopsy-proven liver fibrosis in the pre-ESA era^{6,8,9}.

In the current study, 34.5%, 20%, and 45.5% of the patients showed mild, moderate, or severe liver siderosis, respectively. The patients showed higher serum ferritin (3531 $\mu\text{g/l}$) and TSAT (77%) than other studies due to iron deficiency overtreatment (weekly iron dose 252 mg) and infrequent follow-ups of iron status. A study reported hepatic and splenic siderosis in 19/21 patients with a mean serum ferritin of 2688 ± 1489 ng/mL and a TSAT of $54.2 \pm 32.7\%$ ⁴.

Adhering to target ferritin levels recommended by guidelines did not impact the incidence of liver siderosis in HD patients in studies, in which HD patients were treated with intravenous iron to a target of 200-500 $\mu\text{g/l}$ according to the KDOQI-2006 guidelines and the EBPG statement^{5,19,20}. Surprisingly, hepatic iron overload was shown in 84% of 119 HD patients, 35.3%, 18.5%, and 30.2% of whom showed mild, moderate, or severe liver siderosis, respectively. These findings indicate poor association of serum ferritin with the LIC.

Interestingly, we found that all patients with severe liver siderosis showed advanced liver fibrosis. Patients with severe liver siderosis and advanced liver stiffness also showed higher serum iron, TSAT, AST, ALT, serum bilirubin, APRI, and Fib-4 index scores, and lower total serum protein and serum albumin than patients with mild liver siderosis and mild liver fibrosis. In accordance with this, a study on sickle cell anemia patients showed a strong correlation between LSM and MRI T2*. Moreover, LSM also showed a strong correlation with biochemical indicators of liver function

²¹. Our findings support the speculation that liver fibrosis might be the endpoint of severe liver siderosis in HD patients with severe hyperferritinemia and high iron doses. The scarcity of post-mortem biopsy-proven liver fibrosis reported in some studies might have been associated with shorter durations of liver siderosis ⁶⁻⁹.

We found that only serum iron showed an independent association with the degree of liver fibrosis. Serum ferritin showed no difference between the mild and advanced liver fibrosis groups and among the mild, moderate, and severe liver siderosis groups. In contrast to this, Altunören et al. reported an association between the mean Fibroscan value and the serum ferritin ²². This discrepancy might be attributed to the huge differences in the mean ferritin levels between the two studies. Adhoute et al. conducted a study on hereditary hemochromatosis patients, where they showed no significant correlation between serum ferritin and LSM ²³. They found, in contrast, a significant correlation between the LSM and biochemical markers of liver fibrosis ¹⁴.

A recent study in HFE-associated hemochromatosis revealed that the APRI and Fib-4 index scores were significantly associated with the biopsy-proven liver fibrosis stage. A cut-off Fib-4 score above 1.1 discriminated advanced liver fibrosis with a sensitivity of 80% and a specificity of 80.3%. Likewise, APRI-scores above 0.44 discriminated advanced liver fibrosis with a sensitivity of 79.4% and a specificity of 79.4% ¹¹. In the current study, we used these cut-offs of Fib-4 and APRI scores as surrogates for advanced liver fibrosis. LSM estimated with FibroScan demonstrated 78% sensitivity and 99.95% specificity for detection of advanced liver cirrhosis in HD patients using a cut-off value of 8.9 kPa in reference to APRI, and 69% sensitivity and 89% specificity with a cut-off value of 8.65 kPa in reference to Fib-4 score.

Serum iron and TSAT demonstrated a good utility in discriminating advanced from mild liver fibrosis determined with FibroScan (serum iron: cut-off 153 µg/dl, 87.5% sensitivity, 58.6

specificity; TSAT: cut-off 81%, 67% sensitivity, 76% specificity), Fib-4 (serum iron: cut-off 153 µg/dl, 80 % sensitivity, 73% specificity; TSAT: cut-off 76.5%, 69% sensitivity, 78% specificity) and APRI scores (serum iron: 176.5 µg/dl, sensitivity 61%, 82% specificity; TSAT: cut-off 76.5%, 61% sensitivity, 82% specificity). Similarly, serum ferritin proved to be useful for diagnosis of advanced liver fibrosis determined with Fib-4 (cut-off 1575 µg/l, 97% sensitivity, 56% specificity) and APRI scores (cut-off 1575 µg/l, 97% sensitivity, 45% specificity).

Our study has limitations. Firstly, the diagnosis and staging of liver fibrosis were not histologically confirmed with a liver biopsy. Secondly, despite its importance, the exact duration of iron administration could not be retrieved due to lack of accurate registration. Lastly, the number of recruited patients was relatively small.

In conclusion, high weekly intravenous iron dose associated with severe hyperferritinemia, high serum iron, and TSAT might lead to severe liver siderosis and concomitant liver fibrosis in HD patients. Serum iron and TSAT, Fibroscan, Fib-4 and APRI scores might offer non-invasive tools for identifying advanced liver fibrosis in those patients.

Practical application

Intravenous iron should be administered cautiously and under regular monitoring of iron parameters in HD patients. Fibroscan might be used as a valuable tool to identify advanced liver fibrosis in HD patients. Fibroscan might be warranted in HD patients with chronically high serum iron and TSAT.

Abbreviations

ALP: alkaline phosphatase

ALT: alanine aminotransferase

APRI: Aspartate aminotransferase-to-platelet ratio index

AST: aspartate aminotransferase

BALP: bone alkaline phosphatase

CKD: chronic kidney disease

ESKD: end-stage kidney disease

Fib-4: fibrosis-4 index

HD: hemodialysis

kPa: kilopascal

LSM: liver stiffness measurement

TIBC: total iron binding capacity

TSAT: transferrin saturation

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Figure legends

Figure 1. Area under the curves (AUCs) of receiving operator characteristics for serum iron, TSAT, serum ferritin, and TIBC as possible identifiers of advanced liver fibrosis (LSM >9.5 kPa). The AUC of the serum iron and TSAT showed statistical significance (AUC = 0.804, $p < 0.001$, AUC = 0.71, $p = 0.009$, respectively). AUC values of serum ferritin and TIBC (dashed lines) was not significantly increased (AUC = 0.643, $p = 0.075$; AUC = 0.580, $p = 0.321$, respectively).

Figure 2. A) Area under the curves (AUCs) of receiving operator characteristics for serum iron, TSAT, serum ferritin, and TIBC as possible identifiers of advanced liver fibrosis (Fib-4 >1.1). The AUC of the serum iron, TSAT, and serum ferritin showed statistical significance (AUC = 0.778, $p = 0.001$, AUC = 0.737, $p = 0.005$, AUC = 0.689, $p = 0.025$, respectively). AUC value of TIBC (dashed line) was not significantly increased (AUC = 0.651, $p = 0.173$). **B)** Area under the curves (AUCs) of receiving operator characteristics for serum iron, TSAT, serum ferritin, and TIBC as possible identifiers of advanced liver fibrosis (APRI > 0.44). The AUC of the serum iron, TSAT, and serum ferritin showed statistical significance (AUC = 0.777, $p = 0.001$, AUC = 0.725, $p = 0.006$, AUC = 0.717, $p = 0.008$, respectively). AUC value of TIBC (dashed line) was not significantly increased (AUC = 0.585, $p = 0.295$).

Figure 3. Area under the curves (AUCs) of receiving operator characteristics for LSM (kPa) as a possible identifier of advanced liver fibrosis (APRI > 0.44). The AUC of the LSM showed statistical significance (AUC = 0.834, $p < 0.001$).

Supplementary Figures

Supplementary Figure 1. Area under the curves (AUCs) of receiving operator characteristics for LSM (kPa) as a possible identifier of advanced liver fibrosis (Fib-4 > 1.1). The AUC of the LSM showed statistical significance (AUC = 0.776, $p = 0.001$).

Tables

Table 1. Baseline clinical and laboratory characteristics of the study patients

	HD patients			p-value
	Total (n=55)	Mild liver fibrosis (n=30)	Advanced liver fibrosis (n=25)	
Age	44 ± 12	43.5 ± 13	44.6 ± 11	0.735
Sex (n, % of males)	29 (52.7%)	14 (46.7%)	15 (60%)	0.134
Hypertension (n, %)	31 (56.4%)	16 (53.3%)	15 (60%)	0.191
Diabetes mellitus (n, %)	15 (27.3%)	7 (23.3%)	8 (32%)	0.185
BMI (kg/m ²)	24.8 ± 3.7	24.9 ± 4	24.7 ± 3	0.577
Dialysis duration (years)	6 ± 2	5.7 ± 1.8	6.5 ± 2	0.169
Total number of blood transfusions since dialysis	3 (2-5)	2 (2-4)	5 (3-6)	<0.001
Weekly intravenous erythropoietin alpha dose (IU)		6000 (4000 -8000)	10000 (8000 – 12000)	<0.001
Weekly iron dose (mg)	252.7 ± 63	246.6 ± 68	260 ± 57.7	0.5
AST (u/l)	35 (26 – 63)	26.5 (22 – 33)	63 (45 – 80)	<0.001
ALT (u/l)	51 (33 – 70)	34 (28 – 41)	70 (55 – 78)	<0.001
ALP (u/l)	350 (200 – 500)	243 (184 – 387)	342 (243 – 579)	0.018

Total bilirubin (μmol/l)	7 (5 – 14)	6 (5 – 8)	13 (7 – 28)	<0.001
Serum albumin (g/dl)	37 (33 – 40)	39 (36 – 42)	33 (30 – 39)	<0.001
Total serum protein (g/dl)	67 (57 – 73)	70 (66 – 75)	57 (50 – 68)	0.001
Serum collagen IV (pg/ml)	6013 (3000 – 9555)	4508 (3144 – 7945)	6998 (2904 – 12294)	0.220
Hemoglobin (g/dl)	9.6 (9 – 11)	9.8 (9 – 11)	9.5 (8.5 – 11)	0.446
Platelets (10³/μl)	180 (127 – 222)	206 (179 – 265)	126 (110 – 157)	<0.001
MCV (fl)	88 (85 – 90)	88 (85 – 90)	88 (82 – 90)	0.677
CRP (mg/l)	7 (4 – 17)	7 (4 – 17)	7 (3 – 20)	0.973
Serum iron (μg/dl)	170 (123 – 186)	138 (99-178)	181 (165-207)	<0.001
Serum ferritin (μg/l)	3531 (1716 – 5281)	2995 (1459 - 5155)	4051 (2352 – 5653)	0.083
TSAT (%)	77 (49 – 90)	64 (44 – 86)	86 (68 -93)	0.008
TIBC (μg/dl)	200 (186 – 236)	199 (172 – 239)	208 (190 – 239)	0.526
Fibroscan value (kpa)	8.5 (6-15)	6.1 (4.8-7.9)	17 (10-27.5)	<0.001
APRI score	0.5 (0.3 – 1)	0.3 (0.2 – 0.5)	1 (0.8 – 1.6)	<0.001
Fib-4 score	1.38 (0.7 – 2.4)	0.96 (0.6 – 1.3)	2.48 (1.6 – 3.7)	<0.001

ALP: alkaline phosphatase, BMI: body mass index, CRP: C-reactive protein, HD: hemodialysis, MCV: mean corpuscular volume, fl: femtoliter, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TSAT: transferrin saturation, TIBC: total iron-binding capacity, kpa: kilopascal; APRI: Aspartate aminotransferase-to-platelet ratio index; Fib-4: Fibrosis-4 Index.

Table 2. Comparison of biochemical indicators of iron status and liver function in reference to the degree of liver siderosis

	Mild liver siderosis (n =19)	Moderate liver siderosis (n = 11)	Severe liver siderosis (n = 25)	P0	P1	P2	P3
Serum iron (µg/dl)	135 (81-175)	163 (110-185)	181 (165-207)	0.001	0.860	0.110	0.001
Serum ferritin (µg/l)	2500 (1477-5115)	3572 (1406-5276)	4051 (2352-5653)	0.222			
TSAT (%)	70 (40-87)	63 (49-80)	86 (68-93)	0.026	1	0.276	0.029
TIBC (µg/dl)	200 (165-250)	199 (172-235)	208 (190-239)	0.814			
ALT (u/l)	32 (24-37)	41 (34-60)	67 (55-78)	<0.001	0.091	0.057	<0.001
AST (u/l)	26 (19-32)	32 (23-45)	63 (45-80)	<0.001	0.71	0.002	<0.001
ALP (u/l)	260 (120-374)	231 (200-643)	342 (243-579)	0.05			
Serum albumin (g/dl)	39 (34-42)	39 (37-44)	33 (30-39)	0.002	1	0.043	0.003
Total serum protein (g/dl)	70 (66-73)	74 (65-76)	57 (50-68)	0.002	1	0.006	0.019
Serum bilirubin (µmol/l)	5 (5-7)	7 (5-8)	13 (7-28)	<0.001	0.515	0.084	<0.001
Serum collagen IV (pg/ml)	4425 (3000-9504)	4591 (3192-6955)	6998 (2904-12294)	0.471			
Liver cirrhosis (n, %)	0 (0%)	0 (0%)	10 (18.2%)	<0.001			

APRI score	0.3 (0.2-0.4)	0.4 (0.2-0.6)	1 (0.8-1.6)	<0.001	1	0.007	<0.001
Fib-4 score	0.78 (0.5-1.2)	1.2 (0.68-1.8)	2.5 (1.6-3.7)	<0.001	0.767	0.034	<0.001

P0 value compares mild, moderate, and severe liver hemochromatosis, *P1* value compares between mild and moderate liver hemochromatosis, *P2* value compares between moderate and severe liver hemochromatosis, *P3* value compares between mild and severe hemochromatosis, Bold numbers refer to statistically significant differences.

Abbreviations: ALP: alkaline phosphatase, BMI: body mass index, CRP: C-reactive protein, HD: hemodialysis, MCV: mean corpuscular volume, fl: femtoliter, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TSAT: transferrin saturation, TIBC: total iron-binding capacity, kpa: kilopascal; APRI: Aspartate aminotransferase-to-platelet ratio index; Fib-4: Fibrosis-4 Index.

Table 3. Comparison between patients with non-invasively diagnosed mild and advanced liver fibrosis based on APRI and Fib-4 scores according to the cut-offs of Fibroscan scores that provided the best sensitivity and specificity

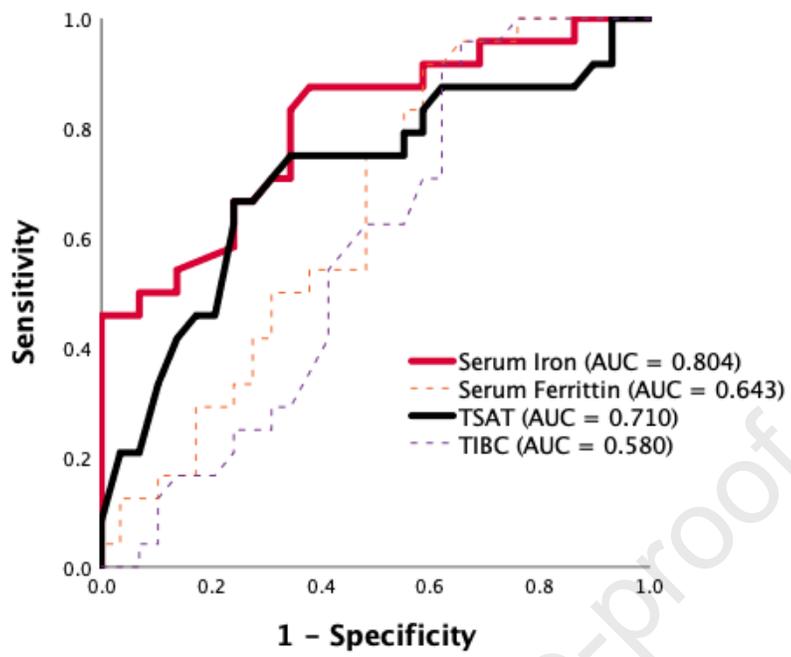
	Fib-4			APRI score		
	Mild liver fibrosis	Advanced liver fibrosis	<i>p</i> value	Mild liver fibrosis	Advanced liver fibrosis	<i>p</i> value
Serum iron (µg/dl)	135 (73.5-173)	178 (158-190)	0.043	138 (89-175)	180 (149-197)	0.053
TSAT (%)	61 (40-77)	86 (57-92)	0.011	63 (40-80)	86 (57-92)	0.017
Serum ferritin (µg/l)	1500 (1081-5115)	3653 (2246-5632)	0.569	2200 (1128-4560)	4413 (2387-6350)	0.271
TIBC (µg/dl)	200 (165-236)	204 (190-244)	0.784	200 (172-236)	204 (190-244)	0.838
AST (u/l)	25 (19-30)	54 (34.7-72)	<0.001	25 (22-30)	55 (43-76)	<0.001
ALT (u/l)	34 (26-39)	61 (41-76.7)	<0.001	33 (24-39)	65 (50-78)	<0.001
ALP (u/l)	266 (199-428)	305 (216-500)	0.639	260 (175-428)	318 (231-558)	0.327
Serum bilirubin (µmol/l)	7 (5-9)	9 (6-23)	0.109	5 (5-8)	11 (7-23.5)	0.009
Total serum protein (g/dl)	70 (67-74)	62 (51-72)	0.018	70 (66-74)	60 (50-72)	0.021

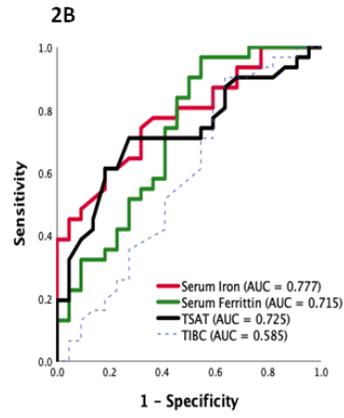
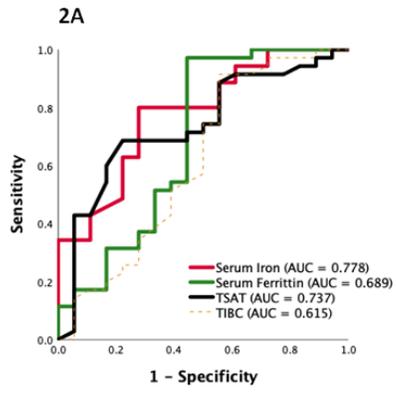
Serum albumin (g/dl)	39 (37-41)	34 (30-40)	0.018	39 (37-42)	33 (30-39)	0.021
Serum collagen IV (pg/ml)	3476 (2765-7512)	6512 (3172-11699)	0.3	5432 (3192-9247)	6464 (2969-10878)	0.665

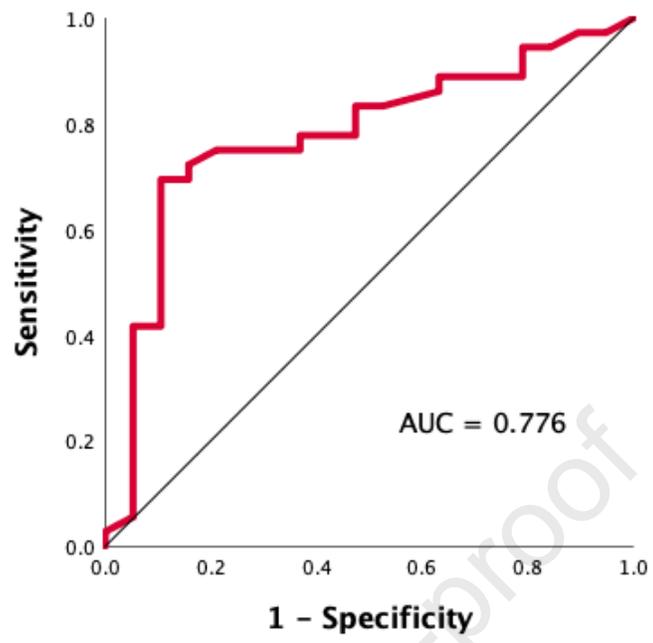
Bold numbers refer to statistically significant differences

Abbreviations: ALP: alkaline phosphatase, BMI: body mass index, CRP: C-reactive protein, HD: hemodialysis, MCV: mean corpuscular volume, fl: femtoliter, AST: aspartate aminotransferase, ALT: alanine aminotransferase, TSAT: transferrin saturation, TIBC: total iron-binding capacity, kpa: kilopascal; APRI: Aspartate aminotransferase-to-platelet ratio index; Fib-4: Fibrosis-4 Index.

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Authors' contributions

MGA, WI; and MMA designed the study. SA conducted all radiological investigations the study, whereas DN performed all laboratory tests needed in the study. MMA recruited the study patients. MGA conducted the statistical analysis. MGA, WI, and MMA wrote the manuscript. DN, SA, and AK made substantial contributions to conception and design of the study. DN, AK, and SA critically revised the manuscript. AK, DN, and SA have been involved in important intellectual content. All authors have given final approval of the manuscript to be published.