ORIGINAL RESEARCH

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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Objective: 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 (LSMs) in identifying advanced liver fibrosis. We investigated the performance of biochemical indicators of iron status in identifying advanced liver fibrosis.

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

Results: The median serum ferritin and transferrin saturation (TSAT) were 3531 μg/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, aspartate aminotransferase (AST), alanine aminotransferase (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, TSAT, Fibroscan, Fib-4, and APRI scores might offer noninvasive 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. The safety of long-term iron administration in dialysis patients has been controversially discussed.

Magnetic resonance imaging (MRI) is the tool of choice for the 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 the 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 postmortem biopsy-proven liver fibrosis in the pre-ESA era.[6,8,9]

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A 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 aminotransferase-to-platelet ratio index 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 the 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 HD 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 the diagnosis of iron overload (serum iron, serum ferritin, transferrin saturation (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 on regular HD for at least 2 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-dependentCKD patients (NDD-CKD), hepato-renal syndrome as the cause of end-stage kidney disease, 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 nonalcoholic 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 TSAT, and serum ferritin.
- Estimation of the biochemical indicators of liver function, including platelets, AST, alanine aminotransferase (ALT), serum bilirubin, serum total protein, and serum albumin.
- Calculation of noninvasive panel scores for liver fibrosis, including the APRI and the fibrosis-4 Index (Fib-4) as follows:

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

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

- 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 : 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 MRI: MRI measurements were performed with a Philips Achieva 1.5 T 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 1 week without iron administration elapsed before conducting MRI. Three MRI methods were applied to estimate LIC qualitatively; signal intensity ratio according to Rennes University,[15] R2* relaxometry, and R2* relaxometry with multiple 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 the 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 institutional review board 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 ± standard deviation, or as the median and interquartile range. 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 the Spearman rank correlation test. Categorical variables were compared with the Chi-square test. Univariable and multivariable logistic regression analyses with the backward elimination method were conducted. Independent variables with significant associations in the univariable analysis ($P < .1$) 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 index.[17] 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 HD regimen consisted of 3 dialysis sessions per week, each lasting 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 (interquartile range [2-5]). Patients received blood transfusions whenever their hemoglobin level was below 7 g/dL. The 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.

Liver Iron Concentration in Hemodialysis 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 (45.5%) liver siderosis groups. 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 ($P = .001$; $P = .026$, respectively). Post-hoc comparisons demonstrated that the serum iron and TSAT were significantly higher in the severe than the mild liver siderosis group ($P = .001$; $P = .029$, 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 ($P = .222$; $P = .814$, respectively) (Table 2).

Biochemical Indicators of Liver Function in Relation to the Liver Iron Concentration

ALT, AST, ALP, serum bilirubin, serum total protein, serum albumin, APRI, and Fib-4 index scores were classified according to the LIC estimated by an MRI scan. ALT and serum bilirubin were significantly higher in the severe liver siderosis as compared to the mild liver siderosis group ($P < .001$, $P < .001$). ALT was significantly higher in the severe liver siderosis group compared with the low and moderate siderosis groups ($P < .001$, $P = .002$, respectively). Both serum total protein and serum albumin were significantly lower in the severe than in the mild and moderate liver siderosis groups ($P = .019$, $P = .006$; $P = .003$, $P = .043$, respectively). Although ALP was comparable among the 3 liver siderosis groups ($P = .05$), all groups showed higher than average ALP levels. This might be in part attributed to an increase in bone alkaline phosphatase. Unfortunately, bone alkaline phosphatase was not estimated in the current study. Patients with severe liver siderosis showed significantly higher APRI and Fib-4

Table 1. Baseline Clinical and Laboratory Characteristics of the Study Patients

	HD Patients			
	Total (n = 55)	Mild Liver Fibrosis (n = 30)	Advanced Liver Fibrosis (n = 25)	*P*-Value
Age	44 ± 12	43.5 ± 13	44.6 ± 11	.735
Sex (n, % of males)	29 (52.7%)	14 (46.7%)	15 (60%)	.134
Hypertension (n, %)	31 (56.4%)	16 (53.3%)	15 (60%)	.191
Diabetes Mellitus (n, %)	15 (27.3%)	7 (23.3%)	8 (32%)	.185
Body Mass Index (kg/m^2)	24.8 ± 3.7	24.9 ± 4	24.7 ± 3	.577
Dialysis Duration (years)	6 ± 2	5.7 ± 1.8	6.5 ± 2	.169
Total Number of Blood Transfusions Since Dialysis	3 (2-5)	2 (2-4)	5 (3-6)	**<.001**
Weekly Intravenous Erythropoietin Alpha Dose (IU)		6000 (4000 -8000)	10000 (8000-12000)	**<.001**
Weekly Iron Dose (mg)	252.7 ± 63	246.6 ± 68	260 ± 57.7	.5
AST (u/L)	35 (26-63)	26.5 (22-33)	63 (45-80)	**<.001**
ALT (u/L)	51 (33-70)	34 (28-41)	70 (55-78)	**<.001**
ALP (u/L)	350 (200-500)	243 (184-387)	342 (243-579)	**.018**
Total Bilirubin (μmol/L)	7 (5-14)	6 (5-8)	13 (7-28)	**<.001**
Serum Albumin (g/dL)	37 (33-40)	39 (36-42)	33 (30-39)	**<.001**
Total Serum Protein (g/dL)	67 (57-73)	70 (66-75)	57 (50-68)	**.001**
Serum Collagen IV (pg/mL)	6013 (3000-9555)	4508 (3144-7945)	6998 (2904-12294)	.220
Hemoglobin (g/dL)	9.6 (9-11)	9.8 (9-11)	9.5 (8.5-11)	.446
Platelets (10^3/μL)	180 (127-222)	206 (179-265)	126 (110-157)	**<.001**
MCV (fL)	88 (85-90)	88 (85-90)	88 (82-90)	.677
CRP (mg/L)	7 (4-17)	7 (4-17)	7 (3-20)	.973
Serum Iron (μg/dL)	170 (123-186)	138 (99-178)	181 (165-207)	**<.001**
Serum Ferritin (μg/L)	3531 (1716-5281)	2995 (1459-5155)	4051 (2352-5653)	.083
TSAT (%)	77 (49-90)	64 (44-86)	86 (68 -93)	**.008**
TIBC (μg/dL)	200 (186-236)	199 (172-239)	208 (190-239)	.526
Fibroscan Value (kpa)	8.5 (6-15)	6.1 (4.8-7.9)	17 (10-27.5)	**<.001**
APRI Score	0.5 (0.3-1)	0.3 (0.2-0.5)	1 (0.8-1.6)	**<.001**
Fib-4 Score	1.38 (0.7-2.4)	0.96 (0.6-1.3)	2.48 (1.6-3.7)	**<.001**

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

Bold *P* values denote statistically significant differences between compared groups.

index scores than those with mild or moderate liver siderosis (APRI: $P < .001$, $P = .007$, respectively; Fib-4: $P < .001$, $P = .034$, respectively). We found no difference in the serum collagen IV levels among the 3 liver siderosis groups (Table 2).

Liver Stiffness Estimated with Fibroscan in Hemodialysis patients

The median liver stiffness value was 8.5 kpa. HD patients were divided into 2 groups according to their LSM; patients with values ≤ 9.5 kPa (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 doses than those with mild liver fibrosis ($P < .001$, $P < .001$, respectively). Moreover, the advanced liver fibrosis group showed higher ALT ($P < .001$), AST ($P < .001$), ALP ($P = .018$), total bilirubin ($P < .001$), whereas it exhibited lower total serum proteins, serum albumin, and platelet concentration ($P < .001$; $P = .001$; $P < .001$, respectively). In addition, the advanced liver fibrosis group showed higher serum iron and TSAT than the mild liver fibrosis group ($P < .001$; $P = .008$, respectively). Neither the serum ferritin nor the TIBC differed significantly between the 2 groups ($P = .083$; $P = .526$, 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 Liver Iron Concentration (Assessed With Magnetic Resonance Imaging) in Hemodialysis Patients

We investigated the relationship between the grade of liver siderosis (assessed with an MRI scan) and the severity of liver fibrosis (estimated with Fibroscan). A significant association could be detected (Chi-square test, $P < .001$). 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.

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)	*P*0	*P*1	*P*2	*P*3
Serum Iron (μg/dL)	135 (81-175)	163 (110-185)	181 (165-207)	**.001**	.860	.110	**.001**
Serum Ferritin (μg/L)	2500 (1477-5115)	3572 (1406-5276)	4051 (2352-5653)	.222			
TSAT (%)	70 (40-87)	63 (49-80)	86 (68-93)	**.026**	1	.276	**.029**
TIBC (μg/dL)	200 (165-250)	199 (172-235)	208 (190-239)	.814			
ALT (u/L)	32 (24-37)	41 (34-60)	67 (55-78)	**<.001**	.091	.057	**<.001**
AST (u/L)	26 (19-32)	32 (23-45)	63 (45-80)	**<.001**	.71	**.002**	**<.001**
ALP (u/L)	260 (120-374)	231 (200-643)	342 (243-579)	**.05**			
Serum Albumin (g/dL)	39 (34-42)	39 (37-44)	33 (30-39)	**.002**	1	**.043**	**.003**
Total Serum Protein (g/dL)	70 (66-73)	74 (65-76)	57 (50-68)	**.002**	1	**.006**	**.019**
Serum Bilirubin (μmol/L)	5 (5-7)	7 (5-8)	13 (7-28)	**<.001**	.515	.084	**<.001**
Serum Collagen IV (pg/mL)	4425 (3000-9504)	4591 (3192-6955)	6998 (2904-12294)	.471			
Liver Cirrhosis (n, %)	0 (0%)	0 (0%)	10 (18.2%)	**<.001**			
APRI Score	0.3 (0.2-0.4)	0.4 (0.2-0.6)	1 (0.8-1.6)	**<.001**	1	**.007**	**<.001**
Fib-4 Score	0.78 (0.5-1.2)	1.2 (0.68-1.8)	2.5 (1.6-3.7)	**<.001**	.767	**.034**	**<.001**

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

P0 value compares mild, moderate, and severe liver hemochromatsis, *P*1 value compares between mild and moderate liver hemochromatosis, *P*2 value compares between moderate and severe liver hemochromatosis, *P*3 value compares between mild and severe hemochromatosis. Bold numbers refer to statistically significant differences.

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, $P = .003$) (Table S1). 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: OR 1.020, 95% CI 1.004-1.036, $P = .015$; mild liver siderosis in reference to APRI score: OR 0.113, 95% CI 0.023-0.566, $P = .008$) (Table S2).

Reliability of Iron Parameters in Discriminating Advanced Liver Fibrosis in Hemodialysis 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 an ROC analysis. When the liver fibrosis grade estimated with Fibroscan was used as the reference standard, the serum iron provided the highest area under curve (AUC) (0.804, $P < .001$; cut-off value 153 μg/dL; sensitivity 87.5%; specificity 58.6%), followed by TSAT (AUC = 0.710, $P = .009$; cut-off value 81%; sensitivity 67%; specificity 76%). Both serum ferritin and TIBC were not valid to detect advanced liver stiffness (AUC = 0.643, $P = .075$; AUC = 0.580, $P = .321$) (Fig. 1). When the 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, $P = .001$; cut-off value 153 μg/dL; sensitivity 80%, specificity 72%), followed by TSAT (AUC = 0.737, $P = .005$; cut-off value 76.5%; sensitivity 69%; specificity 78%) and serum ferritin (AUC = 0.689, $P = .025$; cut-off value 1575 μg/L; sensitivity 97%, specificity 56%) (Fig. 2A). Similarly, the APRI score was dichotomized (≤0.44 vs. > 0.44) and used as the reference standard. Serum iron showed the highest AUC (0.777, $P = .001$; cut-off value 176.5 μg/dL; sensitivity 61%, specificity 82%), followed by TSAT (AUC = 0.725, $P = .006$; cut-off value 76.5%; sensitivity 71%, specificity 73%) and serum ferritin (AUC = 0.717, $P = .008$; cut-off value 1575 μg/L; sensitivity 97%; specificity 45%) (Fig. 2B).

Performance of Fibroscan Score in Detecting Advanced Liver Fibrosis Diagnosed With Aminotransferase-to-Platelet Ratio Index and Fib-4 Scores

32 of 55 patients (58.2%) showed APRI scores >0.44, which was shown to be consistent with advanced liver fibrosis,[11] 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 ($P < .001$; 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,

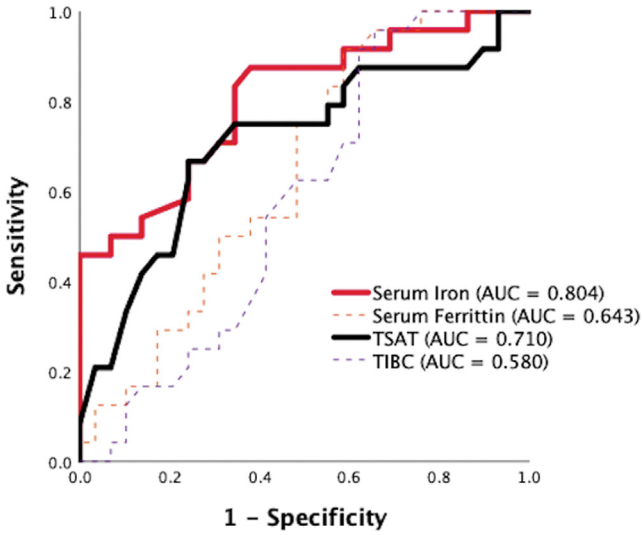

Figure 1. Area under the curves (AUCs) of receiving operator characteristics for serum iron, transferrin saturation (TSAT), serum ferritin, and total iron-binding capacity (TIBC) as possible identifiers of advanced liver fibrosis (liver stiffness measurements (LSM) >9.5 kPa). The AUC of the serum iron and TSAT showed statistical significance (AUC = 0.804, $P < .001$, AUC = 0.71, $P = .009$, respectively). AUC values of serum ferritin and TIBC (dashed lines) were not significantly increased (AUC = 0.643, $P = .075$; AUC = 0.580, $P = .321$, respectively).

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 fibrosis,[11] 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 ($P = .001$; 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

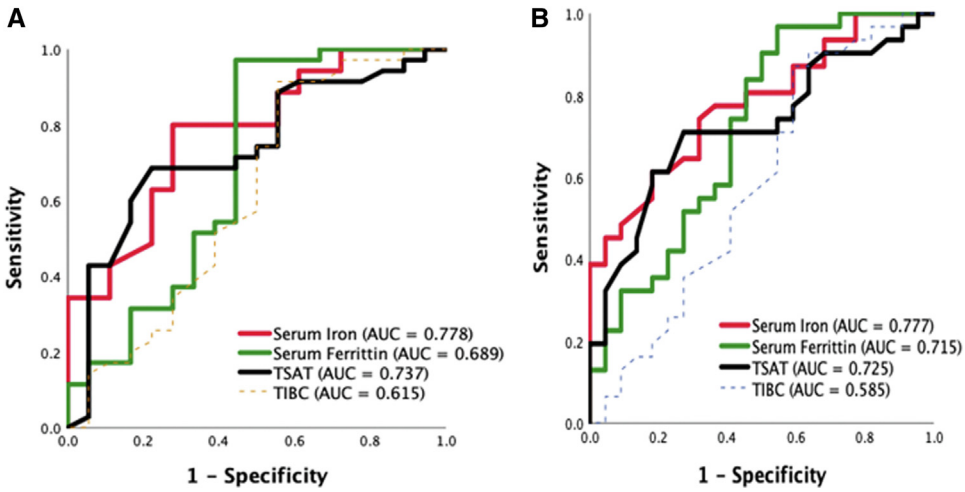

Figure 2. A) AUCs of receiving operator characteristics for serum iron, TSAT, serum ferritin, and TIBC as possible identifiers of advanced liver fibrosis (Fib-4 >1.1). The AUC of the serum iron, TSAT, and serum ferritin showed statistical significance (AUC = 0.778, $P = .001$, AUC = 0.737, $P = .005$, AUC = 0.689, $P = .025$, respectively). AUC value of TIBC (dashed line) was not significantly increased (AUC = 0.651, $P = .173$). B) AUCs of receiving operator characteristics for serum iron, TSAT, serum ferritin, and TIBC as possible identifiers of advanced liver fibrosis (aminotransferase-to-platelet ratio index (APRI) >0.44). The AUC of the serum iron, TSAT, and serum ferritin showed statistical significance (AUC = 0.777, $P = .001$, AUC = 0.725, $P = .006$, AUC = 0.717, $P = .008$, respectively). AUC value of TIBC (dashed line) was not significantly increased (AUC = 0.585, $P = .295$).

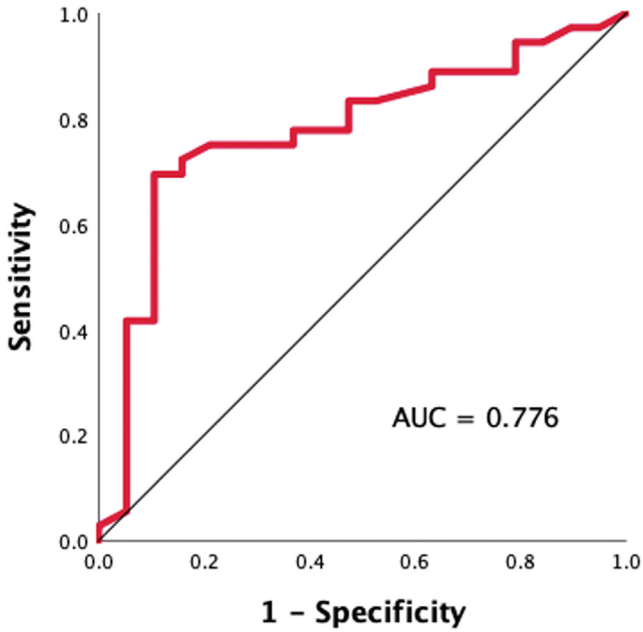

Figure 3. AUCs of receiving operator characteristics for LSM (kPa) as a possible identifier of advanced liver fibrosis (APRI >0.44). The AUC of the LSM showed statistical significance (AUC = 0.834, $P < .001$).

albumin compared with those with Fib-4 scores ≤1.1 kPa (Table 3). Taken together, LSM demonstrates a good utility for the 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 the management of anemia with CKD, iron supplementation should be considered in anemic dialysis patients when TSAT ≤30% and serum ferritin ≤500 ng/ml.[18] Recently, a randomized controlled trial showed that proactive intravenous iron administration in dialysis patients if TSAT ≤40% or serum ferritin ≤700 μg/L was associated with lower frequency of the primary composite endpoint (myocardial infarction, cerebrovascular stroke, hospitalization for heart failure, or death)

Table 3. Comparison Between Patients With Noninvasively 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	*P*-Value	Mild Liver Fibrosis	Advanced Liver Fibrosis	*P*-Value
Serum Iron (μg/dL)	135 (73.5-173)	178 (158-190)	**.043**	138 (89-175)	180 (149-197)	.053
TSAT (%)	61 (40-77)	86 (57-92)	**.011**	63 (40-80)	86 (57-92)	**.017**
Serum Ferritin (μg/L)	1500 (1081-5115)	3653 (2246-5632)	.569	2200 (1128-4560)	4413 (2387-6350)	.271
TIBC (μg/dL)	200 (165-236)	204 (190-244)	.784	200 (172-236)	204 (190-244)	.838
AST (u/L)	25 (19-30)	54 (34.7-72)	**<.001**	25 (22-30)	55 (43-76)	**<.001**
ALT (u/L)	34 (26-39)	61 (41-76.7)	**<.001**	33 (24-39)	65 (50-78)	**<.001**
ALP (u/L)	266 (199-428)	305 (216-500)	.639	260 (175-428)	318 (231-558)	.327
Serum Bilirubin (μmol/L)	7 (5-9)	9 (6-23)	.109	5 (5-8)	11 (7-23.5)	**.009**
Total Serum Protein (g/dL)	70 (67-74)	62 (51-72)	.018	70 (66-74)	60 (50-72)	**.021**
Serum Albumin (g/dL)	39 (37-41)	34 (30-40)	**.018**	39 (37-42)	33 (30-39)	**.021**
Serum Collagen IV (pg/mL)	3476 (2765-7512)	6512 (3172-11699)	.3	5432 (3192-9247)	6464 (2969-10878)	.665

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

Bold numbers refer to statistically significant differences.

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 enzymes[6-8] and the rare association of severe liver siderosis with postmortem 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 μg/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 mean serum ferritin of 2688 ± 1489 ng/mL and a TSAT of 54.2 ± 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 μg/L according to the KDOQI-2006 guidelines and the European Best Practice Guidelines 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 a 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 postmortem 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 2 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 the 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 the lack of accurate registration. Lastly, the number of recruited patients was relatively small.

In conclusion, a 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, TSAT, Fibroscan, Fib-4 and APRI scores might offer noninvasive 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.

Credit Authorship Contribution Statement

Walaa H. Ibrahim: Methodology, Writing – original draft, Final approval for the manuscript to be published. **Marwa M. Abokresha:** Methodology, Recruited the

study patients, Writing – original draft, Final approval for the manuscript to be published. **Dalia A. Nigm:** Performed all laboratory tests needed in the study, Conceptualization, Methodology, Writing – review & editing, Important intellectual content, Final approval for the manuscript to be published. **Sherif M. Abdelal:** conducted all radiological investigations the study, Conceptualization, Methodology, Writing – review & editing, Important intellectual content, Final approval for the manuscript to be published. **Abdalla Kelani:** Conceptualization, Methodology, Writing – review & editing, Important intellectual content, Final approval for the manuscript to be published. **Mostafa G. Aly:** Methodology, conducted the statistical analysis, Writing – original draft, final approval for the manuscript to be published.

Supplementary Data

Supplementary data related to this article can be found at https://doi.org/10.1053/j.jrn.2022.08.004.

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