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ORIGINAL ARTICLE

Circulating Omentin-1 levels and altered iron balance in chronic haemodialysis patients

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ABSTRACT

Background. Iron deficiency is highly prevalent among patients undergoing chronic haemodialysis (HD) but its correct identification is often problematic as common biomarkers of iron status, such as transferrin saturation (TSAT) and ferritin, can be altered by inflammation or malnutrition.

Methods. In this pilot multicentre study, we aimed at evaluating circulating levels of Omentin-1, a novel fat depot-specific adipokine that is also involved in iron regulation, in a cohort of 85 chronic HD patients with relation to their iron status.

Results. Omentin-1 levels in HD were statistically higher than in healthy controls ($P = 0.03$) and there was a significant, growing trend in all iron parameters across Omentin-1 tertiles ($P < 0.001$). Compared with patients with optimal iron status, Omentin-1 levels were lower in subjects categorized according to TSAT ≤20% or serum ferritin ≤200 μg/L (both $P < 0.001$) and even more reduced in 19 patients (22%) simultaneously displaying low levels of both markers ($P < 0.001$). In this latter group, Omentin-1 levels increased in parallel to all other iron markers after iron correction by i.v. supplementation. At multivariate regression analyses, ferritin ($\beta = 0.71$; $P < 0.001$) and TSAT ($\beta = 0.32$; $P = 0.03$) remained the sole independent predictors of Omentin-1 levels. This biomarker also showed a remarkable diagnostic capacity at receiver operating characteristic analyses in identifying iron-depleted HD patients according to a criterion of TSAT ≤20% [area under the curve (AUC) 0.827], ferritin ≤200 μg/L (AUC 0.863) or low levels of both parameters (AUC 0.907).

Conclusions. Findings obtained indicate that Omentin-1 is somewhat involved in iron balance regulation and might be a candidate biomarker for diagnosing and managing altered iron conditions in HD patients.

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VIDEO ABSTRACT

CIRCULATING OMENTIN-1 LEVELS AND ALTERED IRON BALANCE IN CHRONIC HEMODIALYSIS PATIENTS

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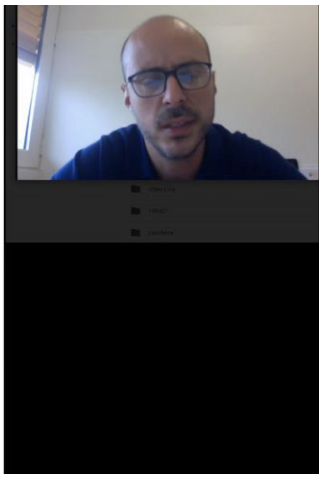

Keywords: ferritin, haemodialysis, iron, Omentin-1, TSAT

INTRODUCTION

A status of iron deficiency is highly prevalent among end-stage kidney disease (ESKD) patients undergoing chronic haemodialysis (HD), mostly as the consequence of a reduced iron absorption and an increased iron loss associated with repeated blood sampling, gastrointestinal bleeding and chronic blood leakage during dialysis. This condition is characterized by severely reduced or absent iron stores in bone marrow, spleen and liver, which leads to chronic anaemia and worsened patient outcomes due to ineffective erythropoiesis [1]. In HD patients, an absolute iron deficit is often revealed by the simultaneous presence of low transferrin saturation (TSAT) (≤20%) and low serum ferritin (≤200 μg/L), which remain two mainstays of iron status assessment and therapy management in this population setting [2, 3]. Nevertheless, the reliability of TSAT and serum ferritin becomes questionable in the presence of 'functional' iron deficiency, a different condition in which normal or even increased total body iron reserves cannot be incorporated into erythroid precursors mostly due to an overall inflammatory state that impairs iron recruitment and its peripheral mobilization [4]. Under these circumstances, the coexistence of low TSAT levels with high or very high serum ferritin levels is not an infrequent finding in HD patients, raising the dilemma of whether starting chronic iron replacement therapy would be an appropriate therapeutic choice [5]. Omentin-1 is a 38-kDa visceral (or omental) fat depot-specific adipokine the main biological function of which is to modulate the effect of insulin on glucose metabolism [6]. Deranged circulating levels of Omentin-1 have predictably been found in various pathological conditions such as insulin resistance, diabetes mellitus, obesity and metabolic syndrome, [7] but interestingly, also in the presence of endothelial dysfunction, atherosclerosis and even osteoporosis [8]. More recently, an anti-inflammatory role for this adipokine has also been postulated in the course of various systemic diseases [9, 10]. Interestingly, a growing body of evidence is now accruing to indicate a key-involvement of Omentin-1 also in physiological iron regulation. Indeed, Omentin-1, which is also known as intelectin-1 or intestinal lactoferrin receptor, is highly expressed on the brush border membrane of the small intestine epithelium with the main function of mediating the uptake of iron-bound lactoferrin and the subsequent internalization by enterocytes via a receptor-mediated endocytosis [11]. Clinically recognized activities of Omentin-1 as the lactoferrin receptor include facilitation of intestinal iron absorption in infants [12] and the strengthening of the immune response to various diseases, presumably by enhancing iron-mediated intracellular pathways consequent to a systemic, targeted iron mobilization [13]. To the best of our knowledge, however, no study has yet placed Omentin-1 in the context of iron alterations that specifically characterize chronic HD patients. We therefore aimed at evaluating Omentin-1 in a pilot cohort of prevalent HD patients, in order to assess any relation this protein may have with the iron balance and its usefulness as a biomarker of iron deficiency.

MATERIALS AND METHODS

Study cohort

We ran a multicentre, observational cohort study on 85 chronic prevalent HD patients undergoing regular dialysis who were recruited from the 'Mater-Domini' University hospital of Catanzaro, Italy (n = 32), the University Hospital of Ioannina, Greece (n = 33) and the General Hospital of Filiates, Greece (n = 20). All patients followed a standard three times/week dialysis rhythm, had a stable dry-weight and an unchanged therapeutic scheme for at least 3 months before entering the study, had achieved a normotensive oedema-free state and had not received iron administration or packed red cell transfusion in the month preceding the start of the study. Exclusion criteria were dialysis vintage <6 months, acute or non-intermittent HD, recent switch from peritoneal dialysis or renal transplantation, presence or a recent history of bleeding, morbid obesity [body mass index (BMI) >40 kg/m^2], malignancy, liver, thyroid or infectious diseases, alterations in leucocyte count or formula and/or treatment with steroids or

immunosuppressors. The local ethic committees approved the study and a fully informed consent was obtained from all participants.

Clinical assessment and Omentin-1 measurement

Anthropometric, clinical and dialysis parameters were recorded before starting a mid-week dialysis session. In addition, common biochemical data were measured, according to standard methods used in the routine clinical laboratory. Iron status was assessed by measuring total serum iron, serum ferritin and TSAT, calculated according to the formula: (serum iron/total iron binding capacity) × 100. Omentin-1 was measured in the blood using an ELISA commercially available kit (Human Intelectin-1/Omentin ELISA Kit, Novus Biologicals, USA), according to the manufacturer's instructions.

The enzymatic reactions were quantified in an automatic microplate photometer and performed in the same laboratory (Clinical Pathology Lab, Mater-Domini' University hospital, Catanzaro, Italy). Measurements were made blind and in duplicate and levels were expressed as ng/mL. Omentin-1 was also tested in a small group of 15 healthy subjects matched with HD patients by gender and BMI.

Iron correction and changes in Omentin-1 levels

After the baseline assessments, long-term changes in Omentin-1 levels were assessed in a sub-cohort of patients showing double evidence of iron deficiency, as documented by the presence of TSAT ≤20% and ferritin ≤200 μg/L. These patients underwent a trial of intravenous (IV) iron replacement therapy by IV sodium ferric gluconate complex given at 177.5 mg (corresponding to 62.5 mg of iron trivalent) each HD treatment for 2 weeks (1065 mg total). Changes in Omentin-1 levels from baseline were therefore assessed in patients achieving and maintaining an optimal iron balance, as defined by the persistence of both TSAT >20% and ferritin >200 μg/L for at least 1 month after the end of the replacement therapy.

Statistical analysis

The statistical analysis was performed using the SPSS (version 24.0.0.0; IBM corporation) package and the MedCalc Statistical Software (version 14.8.1; MedCalc Software bvba). Data were presented as mean ± SD, median [interquartile range (IQR)] or frequency percentage as appropriate. Differences between groups were determined by the unpaired t-test for normally distributed values, the Mann–Whitney U-test for non-parametric values and the chi-square followed by a Fisher's exact test for frequency distributions. Data stratified by tertiles of Omentin-1 were compared using ANOVA for continuous covariates and chi-square for categorical data. A paired t-test and a non-parametric Wilcoxon signed-rank test were employed, as appropriate, to analyse changes in iron parameters and Omentin-1 levels after successful iron correction. The Pearson (R) and the Spearman (Rho) correlation coefficients were employed to test correlations between variables, as indicated. Before testing correlations, all values showing a skewed distribution were log-transformed to better approximate normal distributions. Multiple regression analyses were performed by building a model including all univariate correlates of Omentin-1 in order to assess independent relationships. Data were expressed as partial correlation coefficients (β) and P-value. A receiver operating characteristics (ROC) analysis was employed to calculate the areas under the curve for Omentin-1 for identifying iron-deficient patients according to three different definition models: TSAT ≤20%; serum ferritin ≤200 μg/L and simultaneous presence of TSAT ≤20% and ferritin ≤200 μg/L. The best cut-off values were computed by the Youden index. All results were considered significant if the P-value was <0.05.

RESULTS

Main characteristics of the study cohort

Table 1 depicts the main characteristics of the study cohort. Mean ± SD age of patients was 68 ± 12.7 years and the majority of them were male (72%). The causes of ESKD were nephroangiosclerosis or hypertensive nephropathy (n = 10), glomerulonephritis (n = 23), diabetic nephropathy (n = 8), interstitial nephritis (n = 3), polycystic kidney disease (n = 9) and chronic pyelonephritis (n = 2), and unknown in the remaining (n = 30). Median dialysis vintage was 34 (IQR 17–65.5) months with a satisfactory dialysis adequacy on average (mean Kt: 1.42 ± 0.27). Roughly half of patients (49.4%) were on standard bicarbonate dialysis while the remaining were on hemodiafiltration. The vast majority of individuals were on ESA therapy (90.6%). Twenty-two percent of patients (n = 19) had diabetes, generally displaying a good glycaemic control (mean HbA1c 5.74 ± 1.09). Median levels of Omentin-1 in the whole cohort were 784 (447.6–1519) ng/mL. There was a statistically significant trend across Omentin-1 tertiles in Kt/V values, systolic blood pressure, serum potassium and phosphate, aspartase aminotransferase, alanine aminotransferase, gamma-glutamyl transferase and platelet count (P ranging from 0.05 to <0.001). Omentin-1 levels in HD patients were statistically higher as compared with those measured in healthy controls [228.2 (67.9–366.1) ng/mL; P = 0.03].

Omentin-1 in patients classified according to iron status

The whole cohort presented, on average, a good iron control with mean iron levels of 71.3 ± 27.4 mg/dL, mean TSAT values of 29.8 ± 8.4% and median ferritin levels of 215.9 (IQR 103–357.9) μg/L. A significant, growing trend in all iron parameters was noticed across Omentin-1 tertiles (all P < 0.001; Table 1). Among the study participants, 27/85 individuals (31.7%) were classifiable as iron-deficient because of TSAT values ≤20%. Omentin-1 levels were significantly lower in these individuals as compared with others [391.3 (248.8–573.6) versus 1022 (603.4–1140) ng/mL; P < 0.001; Figure 1A]. With respect to serum ferritin, 46/85 patients (54.1%) showed circulating values below the optimal threshold of 200 μg/L. As observed for TSAT, Omentin-1 levels were significantly reduced also in these patients as compared with the remaining [497 (274.5–748) versus 1568 (791–1830.5) ng/mL; P < 0.001; Figure 1B]. Interestingly, only 19/27 patients with TSAT ≤20% had also serum ferritin values ≤200 μg/L (22.3% of the total cohort), hence fulfilling both lab criteria for iron deficiency. These patients presented even lower Omentin-1 levels as compared with the rest of the study population [286.3 (189–452.2) versus 955.5 (566–1659) ng/mL; P < 0.001; Figure 1C].

Clinical correlates of Omentin-1

At univariate analysis, Omentin-1 was directly correlated with serum ferritin (R = 0.79; P < 0.001), TSAT (R = 0.43; P < 0.001), serum iron (R = 0.40; P < 0.001), aspartate aminotransferase

Table 1. Main clinical characteristics in the whole study cohort and in patients stratified according to tertiles of Omentin-1

	All patients $n = 85$	Omentin-1 (<506.2 ng/mL) $n = 28$	Omentin-1 (506.2–1117.6 ng/mL) $n = 29$	Omentin-1 (>1117.6 ng/mL) $n = 28$	P for trend
Age (years)	68 ± 12.7	69 ± 15.1	68.3 ± 14.9	69.4 ± 13.6	0.88
Males, *n* (%)	61 (72)	20 (71)	21 (72)	20 (71)	0.89
Dry weight (kg)	70.7 ± 15.6	67.3 ± 11.6	72.5 ± 15.2	68 ± 13.2	0.49
BMI (kg/m^2)	25.6 ± 5.6	24.3 ± 4.8	24.8 ± 5.3	26.6 ± 9.8	0.11
Waist-Hip ratio (cm)	0.97 ± 0.11	0.90 ± 0.19	0.93 ± 0.16	0.99 ± 0.13	0.09
Type of dialysis					
Haemodialysis, n (%)	42 (49.4)	16 (57)	12 (41)	14 (50)	0.19
Hemodiafiltration, *n* (%)	43 (51.6)	12 (43)	17 (59)	14 (50)	0.19
Kt/V	1.42 ± 0.27	**1.35 ± 0.33**	**1.38 ± 0.41**	**1.49 ± 0.39**	**0.02**
Dialysis vintage (months)	34 (17–65.5)	18 (8–20)	25 (10–41)	28 (12–50)	0.07
ESA use, *n* (%)	77 (90.6)	25 (89.3)	27 (93.1)	25 (89.3)	0.32
Diabetes, *n* (%)	19 (22)	5 (17.8)	5 (17.2)	9 (32.1)	0.06
HbA1c (%)	5.74 ± 1.09	5.12 ± 1.13	5.32 ± 1.25	6.88 ± 1.23	0.08
Glycaemia (mg/dL)	89.4 ± 16.9	89.5 ± 15.2	84.3 ± 12.1	109 ± 32.3	0.11
Haemoglobin (g/dL)	10.9 ± 1.1	11.2 ± 1.3	10.8 ± 1	11.1 ± 0.9	0.32
SBP (mmHg)	141.5 ± 22.3	**142.3 ± 16.6**	**138.1 ± 16.3**	**133 ± 15.8**	**0.03**
DBP (mmHg)	72.5 ± 11.9	70.9 ± 13.3	74.0 ± 10.9	73.4 ± 12.1	0.08
Serum creatinine (mg/dL)	8.12 ± 2.13	8.10 ± 1.68	8.09 ± 1.95	8.88 ± 2.33	0.46
Urea (mg/dL)	136.8 ± 35.3	141.8 ± 29.1	131.1 ± 34	138.5 ± 39.9	0.38
Sodium (mg/dL)	136.7 ± 13.4	138 ± 12.4	131.8 ± 14.6	141.1 ± 9.9	0.16
Potassium (mg/dL)	4.99 ± 0.70	**5.02 ± 0.45**	**4.75 ± 0.60**	**4.70 ± 0.55**	**0.04**
Phosphate (mg/dL)	4.74 ± 1.39	**5.49 ± 1.12**	**4.44 ± 0.88**	**4.35 ± 1.00**	**0.03**
Calcium (mg/dL)	9.15 ± 0.65	9.08 ± 0.63	9.11 ± 0.55	9.66 ± 0.88	0.48
Magnesium (mg/dL)	2.19 ± 0.51	2.33 ± 0.48	2.46 ± 0.44	2.99 ± 0.5	0.19
iPTH (pg/mL)	231.2(133.3–379.1)	202.4(109.9–388.5)	255.4(163.5–479)	211.9(156–329.4)	0.23
Uric acid (mg/dL)	5.86 ± 1.22	5.45 ± 1.20	5.98 ± 1.36	5.76 ± 1.38	0.35
Albumin (g/dL)	3.94 ± 0.27	3.24 ± 0.33	4.15 ± 0.18	3.90 ± 0.20	0.19
AST (U/L)	12.2 ± 4.2	**9.1 ± 3.2**	**11.1 ± 3.9**	**15.6 ± 1.9**	**0.001**
ALT (U/L)	11.8 ± 4.9	**10.8 ± 3.1**	**10.9 ± 3.9**	**12.1 ± 5.1**	**0.05**
ALP (U/L)	75 (60–89.5)	80.8 (50–92.5)	75.7 (66–88.5)	79.2 (43–86.1)	0.66
GGT (U/L)	16 (11–26)	**12 (8–28)**	**15 (10–30)**	**18 (14–30)**	**0.03**
Total cholesterol (mg/dL)	147.2 ± 43	150.1 ± 38.8	146.3 ± 40	146 ± 36.6	0.08
HDL (mg/dL)	41.8 ± 10.3	44.3 ± 9.9	43.6 ± 10.1	39.9 ± 12.7	0.10
LDL (mg/dL)	82.4 ± 32.2	86.6 ± 28.8	80.2 ± 30.3	85.1 ± 36.6	0.07
Triglycerides (mg/dL)	127 (85–125)	126 (78–156)	129 (90–200)	128 (88–199)	0.66
ESR (mm/h)	30.5 (20–47)	13 (10–85)	48 (25–66)	22 (10–88)	0.39
C-reactive protein (mg/L)	0.7 (0.2–3.23)	3.2 (0.7–35.1)	0.9 (0.3–2.7)	1.9 (0.8–20.8)	0.44
Fibrinogen (mg/dL)	422.7 ± 98.6	426.5 ± 91.1	420.3 ± 88.4	420.7 ± 85.4	0.58
RBC ($n \times 10^3$)	3.79 ± 0.89	3.81 ± 0.69	3.77 ± 0.58	3.98 ± 0.96	0.16
WBC ($n \times 10^3$)	6.92 ± 3.07	6.80 ± 2.77	6.90 ± 3.03	6.67 ± 2.74	0.21
PLT ($n \times 10^3$)	221.3 ± 78	**285 ± 69.1**	**222.4 ± 78.6**	**220.3 ± 80.3**	**0.03**
Serum iron (mg/dL)	71.3 ± 27.4	**39.1 ± 8.3**	**68.3 ± 20.5**	**100.4 ± 27.5**	**<0.001**
TSAT (%)	29.8 ± 8.4	**19.2 ± 6.8**	**24.5 ± 8.0**	**34 ± 7.7**	**<0.001**
Ferritin (μg/L)	215.9 (103–357.9)	**69.6 (30.1–99)**	**116.8 (89–320.2)**	**420.1 (122–575.3)**	**<0.001**
Omentin-1 (ng/mL)	784 (447.6–1519)	**298.6 (186.2–447.6)**	**784 (603.4–925)**	**1711.5 (1519–2075.5)**	**<0.001**

Data are presented as mean ± SD or median (interquartile range), unless otherwise indicated. Statistical significance is highlighted in bold. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartase aminotransferase; DBP, diastolic blood pressure; ESR, erythrocyte sedimentation rate; ESA, erythropoiesis stimulating agents; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PLT, platelet count; iPTH, intact parathormone; RBC, red blood cell count; SBP, systolic blood pressure; WBC, white blood cell count.

(R = 0.24; P = 0.02) and Kt/V (R = 0.23; P = 0.03), whereas significant inverse correlations were observed with serum phosphate (R = −0.31; P = 0.004), systolic blood pressure (R = −0.25; P = 0.02) and platelet count (R = −0.20; P = 0.009). No significant associations were found between Omentin-1 and other clinical, anthropometric and laboratory parameters, including glycaemic levels, presence of diabetes, BMI and waist–hip ratio (R range = −0.06 to 0.68; P > 0.06).

All variables found to be significantly related to Omentin-1 at univariate analysis were introduced in a multivariate model using Omentin-1 as dependent variable. As TSAT represents a function of serum iron, this last variable was not included in the model in order to avoid co-linearity. After adjustment for other factors, significance was maintained only for the correlations between Omentin-1 and, respectively, serum ferritin (β = 0.71; P < 0.001) and TSAT (β = 0.32; P = 0.03), while the correlations with Kt/V, platelets count, aspartate aminotransferase, systolic blood pressure and phosphate, found at univariate analysis, were lost. Of note, this multivariate model resulted remarkably robust, explaining about 72% of the total variance of Omentin-1 in this cohort. Table 2 depicts findings at univariate and multiple regression analysis.

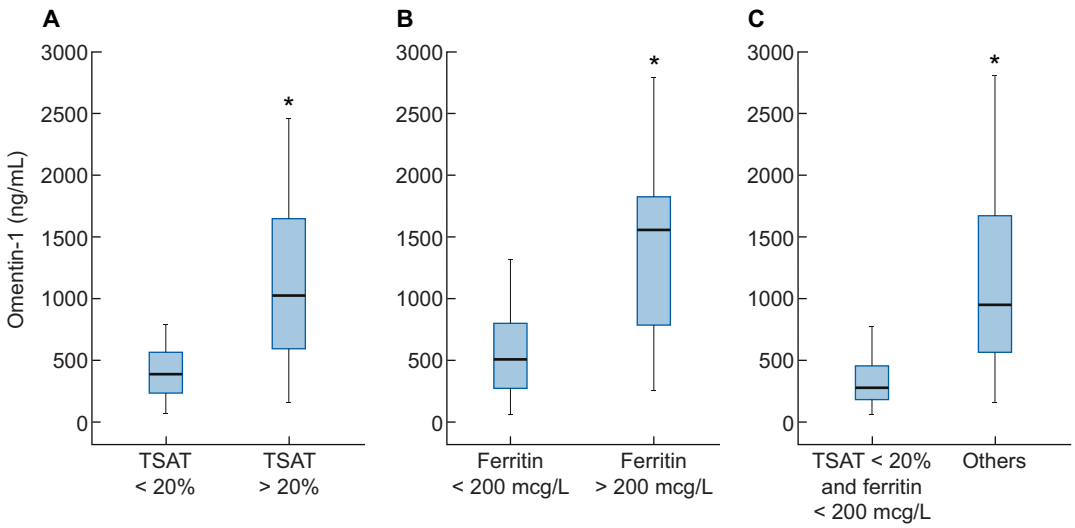

FIGURE 1: Omentin-1 levels in iron-deficient patients categorized according to (**A**) TSAT <20%; (**B**) ferritin <200 μg/L; and (**C**) simultaneous presence of TSAT <20% and ferritin <200 μg/L. *P < 0.001.

Table 2. Univariate and multiple regression analysis of (log-transformed) Omentin-1 levels

	Univariate correlation coefficient	*P*
(Log) Ferritin	0.79	<0.001
TSAT	0.43	<0.001
Serum iron	0.40	<0.001
Phosphate	−0.31	0.004
Systolic blood pressure	−0.25	0.02
Aspartate aminotransferase	0.24	0.02
Kt/V	0.23	0.03
Platelet count	−0.20	0.009
	Multivariate standardized correlation coefficient (β)	*P*
(Log) Ferritin	**0.71**	**<0.001**
TSAT	**0.32**	**0.03**
Kt/V	0.39	0.06
Platelet count	−0.11	0.11
Aspartate aminotransferase	0.10	0.14
Systolic blood pressure	−0.47	0.48
Phosphate	−0.03	0.68

Statistical significance is highlighted in bold. Multiple R = 0.85, R^2 = 72%; P < 0.001.

ROC analysis of Omentin-1 for the identification of iron deficiency

Three different models of ROC analysis were employed to test the diagnostic capacity of Omentin-1 to identify HD patients with iron deficiency. In the first model, we assumed TSAT values ≤20% as the status variable. The area under the curve (AUC) for Omentin-1 to identify patients with iron deficiency in this model was 0.827 [95% confidence interval (CI) 0.721–0.933] with a best cut-off value of 798 ng/mL yielding a sensitivity of 92.5% (95% CI 75.7–99.1) and a specificity of 64.1% (95% CI 49.8–76.9). In the second model, we adopted a ferritin-based criterion (cut-off ≤200 μg/L). The AUC for Omentin-1 was 0.863 (95% CI 0.785–0.940), with a best cut-off of 1029 ng/mL (sensitivity 93.4%, 95% CI 82.1–98.6; specificity 69.2%, 95% CI 52.4–83.0). In the last model we considered as status variable the simultaneous presence of TSAT ≤20% and serum ferritin ≤200 μg/L. The diagnostic performance of Omentin-1 in this last model was the highest reported with an AUC of 0.907 (95% CI 0.844–0.971) and an optimal threshold of 538.3 ng/mL yielding a sensitivity of 86.3% (95% CI 65.1–97.1) and a specificity of 89.2% (95% CI 68.1–90.1). Figure 2 reports findings from ROC analyses.

Effect of iron correction on Omentin-1 levels

Nineteen individuals showing evidence of absolute iron deficiency (TSAT ≤20% and ferritin ≤200 μg/L) underwent a chronic iron supplementation therapy by IV sodium ferric gluconate complex given at 177.5 mg (corresponding to 62.5 mg of iron trivalent) at each HD treatment for 2 weeks (1065 mg total). After this trial, 8/19 patients achieved a successful iron correction with both TSAT and serum ferritin increasing to optimal values (Table 3) that persisted up to 1 month after the end of the therapy. In these individuals, circulating Omentin-1 levels significantly increased from 452 (363.1–621.5) to 1052 (669–1387.2) ng/mL (P = 0.02; Table 3).

DISCUSSION

Findings obtained from our study in chronic HD patients point to Omentin-1 as a new factor potentially involved in iron regulation with a possible clinical application as a biomarker for diagnosing and managing altered iron conditions.

Iron control in HD patients remains a challenging issue. Iron deficiency is frequently found among these subjects, representing a leading cause of refractory anaemia and contributing to the high cardiovascular mortality and morbidity [14, 15].

Serum ferritin and TSAT still remain the primary tools for the assessment of iron status in HD patients [3]. Nevertheless, both parameters are far from being perfect biomarkers of iron storage as ferritin is also an acute-phase reactant whereas TSAT levels might significantly be impacted by malnutrition and, to a lower extent, also by inflammation [16]. As a result, in the last decade, alternative biomarkers have been examined as potential tools to driving iron therapy in HD patients,

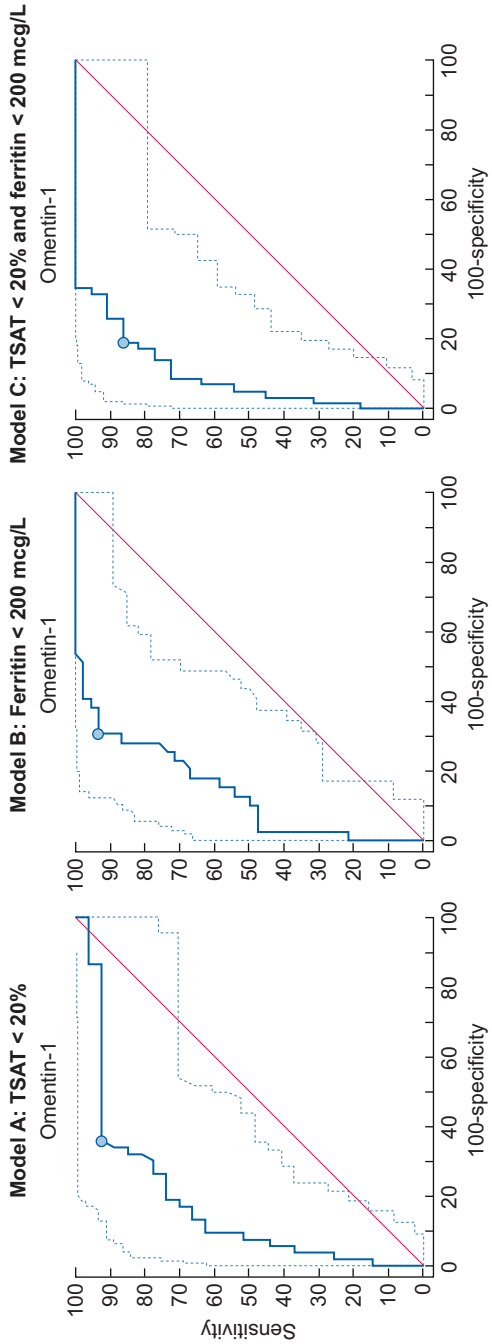

FIGURE 2: AUCs for circulating Omentin-1 considering as status variable for iron deficiency: (model A) TSAT <20%; (model B) serum ferritin <200 μg/L; (model C) a simultaneous condition of TSAT <20% and serum ferritin <200 μg/L. AUCs for Omentin-1 were 0.827 (95% CI 0.721–0.933), 0.863 (95% CI 0.785–0.940) and 0.907 (95% CI 0.844–0.971) for models A, B and C, respectively.

Table 3. Changes in iron parameters and Omentin-1 levels in 19 iron-deficient HD patients after successful iron correction

	Before iron correction	After iron correction	P
Serum iron (mg/dL)	**44.6 ± 11.9**	**79.1 ± 11.8**	**0.04**
TSAT (%)	**17.7 ± 1.1**	**24.3 ± 2.4**	**0.03**
Ferritin (μg/L)	**122.5 (29–131)**	**388 (324–582)**	**0.02**
Omentin-1 (ng/mL)	**452 (363.1–621.5)**	**1052 (669–1387.2)**	**0.02**

Data are presented as mean ± SD or median (interquartile range). Statistical significance is highlighted in bold.

including the soluble transferrin receptor [17], the percentage of hypochromic red blood cells and reticulocyte Hgb content [18], serum hepcidin [19] and the plasmatic levels of the Neutrophil Gelatinase-Associated Lipocalin [20]. Unfortunately, none of these has yet been widely validated, in particular with respect to the capacity of discriminating between a true iron storage deficiency and a dysfunctional iron mobilization due to inflammatory reticulo-endothelial cell iron blockade. This latter, in particular, remains an often under-recognized condition that poses a serious threat to the opportunity to start or halt iron supplementation, balancing the risk of an ineffective correction to an even more dangerous iron overload.

Omentin-1 is a fat depot-specific adipokine belonging to the superfamily of intelectins, a group of glycan-binding lectins that participates in innate immune responses with the ability to agglutinate bacteria [9]. Beyond such an antibacterial activity, more recently, Omentin-1 has also been investigated either as a pathogenic factor and a promising biomarker in various pathological conditions including diabetes, atherosclerosis, chronic heart disease, hypertension, inflammation and neoplasias [6, 21–23]. Of note, various biological functions of Omentin-1 have been acknowledged to depend on its capacity to modulate iron absorption and mobilization, e.g. by binding the lactoferrin as an intestinal receptor or by enhancing iron-mediated intracellular pathways [11, 13].

In our study, we have firstly found increased circulating levels of Omentin-1 in HD patients as compared with healthy subjects. This observation is in line with findings reported from previous studies [24–26] indicating that the chronic renal replacement treatment notably alters the balance of this substance. In these studies, Omentin-1 levels were also found to be influenced by the presence of diabetes [24] and to be potentially useful to predict worsen cardiovascular outcomes and progression of atherosclerosis lesions [25, 26].

Unfortunately, none of these studies apparently collected information on the iron status of the study participants and, consequently, could not evaluate the possible role of Omentin-1 in such regard.

Various findings from our study seem to confirm the growing idea that this protein could be somewhat involved in iron regulation in HD patients. First, a significant, growing trend in all iron parameters was noticed across Omentin-1 tertiles. Second, statistically lower levels of Omentin-1 were observed either in subgroups of patients with TSAT and ferritin below the suggested reference values for starting iron replacement therapy, being even more reduced in a third subgroup of individuals presenting with double evidence of iron depletion (i.e. both TSAT and ferritin below the reference threshold). These observations were further corroborated by results of ROC analyses that confirmed a remarkable diagnostic capacity of Omentin-1 to identify HD patients with iron scarcity, expressed as either

TSAT ≤20% or serum ferritin ≤200 μg/L. Of note, again, such a diagnostic capacity became even more significant in a third model encompassing only individuals with evidence of true absolute iron deficiency (below threshold levels of both TSAT and ferritin simultaneously).

In our study, we have also found strong independent correlations at multivariate regression analysis between Omentin-1 and, respectively, TSAT and serum ferritin levels. Although this discovery cannot fully rule out the impact of residual confounders, the multivariate model implemented resulted in being highly robust and explained a large percentage (72%) of the absolute variability of this substance; this suggests that, in this particular study cohort, iron metabolism remains the stronger determinant of Omentin-1 levels.

Lastly, the correction of iron deficiency with IV iron supplements induced a statistically significant increase in Omentin-1 values, which reached levels very similar to those of HD patients with optimal initial iron storage. Importantly, in order to minimize bias, such longitudinal changes were only ascertained in individuals who presented baseline evidence of absolute iron depletion and who maintained evidence of iron correction up to 1 month after the end of the replacement therapy. Of note, this observation parallels similar findings previously reported for other biomarkers [27], thus indicating that Omentin-1 might represent a potentially useful tool also for the management of iron replacement therapy.

Our study has some limitations that deserve mentioning. First, the study cohort was relatively small. Although robust, our findings would thus need to be externally validated on larger populations. Second, given the observational design, this study cannot clarify whether the deranged Omentin-1 levels represent a simply epiphenomenon or a true pathogenic factor involved in the altered iron equilibrium of HD patients. In addition, the presence of selection bias, significant residual confounding and confounding by indication cannot be fully excluded. Third, despite the multicentre enrolment, the participants were rather homogenous, in particular with respect to dialysis prescription and clinical parameters. No less important, they all showed a good overall nutritional status and a relatively low prevalence of diabetes. As malnutrition is known to potentially alter TSAT values and serum ferritin levels while, on the contrary, diabetes and weight excess represent two strong modulators of Omentin-1 production [23], the impact of different nutritional or glycometabolic statuses on the relationships between this biomarker and iron parameters deserve further examination.

In conclusion, the potential use of Omentin-1 measurement in the assessment of iron status among HD patients remains of great potential, but the findings made in the present study must be considered only preliminary. Further in-depth investigations are eagerly awaited to clarify the exact biological role of Omentin-1 in the regulation of iron balance in HD patients, in order to ascertain at which organ/system level this protein exerts its key regulatory functions. No less important, larger studies are needed to test the clinical applicability of Omentin-1 as a biomarker for identifying abnormal iron conditions, possibly discriminating between absolute and relative iron deficiency, as well as for guiding long-term iron replacement therapy.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request (contact: Davide Bolignano; davide.bolignano@gmail.com).

SUPPLEMENTARY DATA

Supplementary data are available at *ckj* online.

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None declared.

AUTHORS' CONTRIBUTIONS

The research idea was conceived by D.B., G.Crugliano, P.P. and M.A. Data collection and analysis were performed by E.D., A.D., G.Coppolino, C.P., E.P. and L.L. Laboratory measurement was carried out by M.G., F.D. and D.P.F. Manuscript preparation and revision was done by D.B., G.Crugliano, P.P. and E.D.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest with respect to the present work. The results presented in this paper have not been published previously in whole or part, except in abstract format.

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