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Preliminary experience on the use of sucrosomial iron in hemodialysis: focus on safety, hemoglobin maintenance and oxidative stress

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Abstract

Purpose Iron is usually administered in hemodialysis patients by parenteral route, as oral absorption is poor due to high hepcidin levels. However, administrations of intravenous iron and iron overload are associated with high oxidative stress and systemic inflammation that can affect patient survival. With this study, we evaluated an alternative type of oral iron for the treatment of anemia in hemodialysis patients. The formulation consists in ferric pyrophosphate covered by phospholipids plus sucrose ester of fatty acid matrix, named sucrosomial iron, whose absorption is not influenced by hepcidin.

Methods Twenty-four (24) patients undergoing chronic hemodialysis switched iron supplementation from intravenous (ferric gluconate 62.5 mg weekly) to oral (sucrosomial iron, 90 mg weekly in 3 administrations of 30 mg) route for 3 months. Classical anemia, iron metabolism, inflammation and nutritional biomarkers were monitored, as well as biomarkers of oxidative stress, such as protein-bound di-tyrosines, protein carbonylation, advanced oxidation protein products and protein thiols.

Results Over the 3 months, hemoglobin values remained stable, as the values of hematocrit and mean corpuscular volume. In parallel, other anemia parameters dropped, including ferritin, transferrin saturation and serum iron. On the other side, nutritional biomarkers, such as total proteins and transferrin, increased significantly during the time frame. We also observed a significant decrease in white blood cells as well as a non-significant reduction in C-reactive protein and some oxidative stress biomarkers, such as protein carbonyls and di-tyrosines.

Conclusion Our study demonstrates that a therapy with sucrosomial iron in hemodialysis patients is safe and can maintain stable hemoglobin levels in a three-month period with a possible beneficial effect on oxidative stress parameters. However, the reduction of ferritin and transferrin saturation suggests that a weekly dosage of 90 mg is not sufficient in hemodialysis patients in the long time to maintain hemoglobin.

Keywords Iron supplementation · Anemia · Dialysis · Oxidative stress · Sucrosomial iron

Introduction

There are more than 2 million patients on dialysis worldwide and this number is going to increase to more than 5.4 million by 2030 [1]. Most of these patients are anemic because of the relative deficiency of erythropoietin that reduces erythrocytes production [2]. However, a major contribution to anemia in this setting is also played by iron deficiency. As a matter of fact, it is observed in more than 50% of patients with non-dialysis-dependent chronic kidney disease (CKD) and in a greater percentage of patients receiving dialysis. Iron deficiency in dialysis patients is caused by the chronic blood losses due to the extracorporeal treatment and the frequent blood analyses [2]. In addition to this absolute iron deficiency, dialysis patients may also develop functional iron deficiency. In fact, even if iron stores appear to be adequate by conventional criteria, iron cannot be mobilized when erythropoiesis is stimulated by an erythropoiesis stimulating agent (ESA) [3]. This is due to the inability to export the iron ion from macrophages and enterocytes into circulation, as high hepcidin levels due to chronic inflammation

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prevent it by inhibiting ferroportin expression [4]. It has to be highlighted that while daily iron absorption is only 1–2 mg, iron recycling from metabolism of senescent red blood cells is about 20 mg. If ferroportin is unable to release this amount of iron from physiological stores, this highlights the expectations of iron supplementation in this setting. For this reason, a great majority of hemodialysis patients necessitate iron supplementation to a supra-therapeutic level to compensate functional iron deficiency. In the hemodialysis setting, intravenous administration is preferred, since it has been widely demonstrated that intravenous iron determines a greater increase in hemoglobin levels and reduces the need for ESAs [5–7]. The Kidney Disease: Improving Global Outcomes (KDIGO) guidelines suggest a goal-directed intravenous iron replacement when Transferrin Saturation (TSAT) ≤ 30% and serum ferritin ≤ 500 ng/mL [8]. However, Karaboyas et al., analyzing the Dialysis Outcomes and Practice Patterns Study (DOPPS) database, observed a diffuse increase in mean ferritin values, in particular in United States and Europe [9], with possible concerns about the risk of iron overload and patients' outcome. Two different studies have demonstrated an increased mortality in hemodialysis patients treated with higher doses of intravenous iron [10, 11]. Also, a correlation between high ferritin values and mortality has been described [9]. The possible negative effect of intravenous iron and iron overload on mortality may be mediated by oxidative stress. Iron has a high reactivity with oxygen, as described by the Haber–Weiss and Fenton reactions, and brings to the production of hydroxyl radicals, hydrogen peroxide and superoxide. When present in excess within cells and tissues, iron disrupts redox homeostasis and catalyzes the propagation of reactive oxygen species leading to oxidative stress [12]. The human body has highly conserved mechanisms to counteract the production of free radicals secondary to iron metabolism, in which hepcidin has a pivotal role [13, 14]. Injection of iron directly into the bloodstream bypasses these protective controls that evolved to prevent iron overload and thus may determine an increase in oxidative stress [15–20]. Several studies, both in vitro and in vivo, showed an increase of oxidative stress biomarkers after the infusion of intravenous iron, possibly secondary to the increase of the levels of free iron [21–23]. Also a free iron-independent mechanism, suggested by incomplete transferrin saturation after IV iron infusion, has been hypothesized [24]. Oxidative stress is intimately linked to systemic inflammation [25], a strong negative predictor in the hemodialysis setting [26], and this can justify the increased mortality observed when intravenous iron is employed [10, 11].

Sucrosomial iron is a new oral iron preparation containing ferric pyrophosphate covered by phospholipids plus sucrose ester of fatty acid matrix. In contrast to older formulation of oral iron, this new formulation may be a valid alternative to intravenous route in dialysis patients, since its absorption is not influenced by hepcidin levels [27]. To date, in vitro studies have demonstrated that sucrosomal iron is absorbed as a vesicle-like structure, through transcellular and paracellular pathways. In contrast to iron salts that are normally absorbed by enterocytes via DMT-1-dependent mechanisms, sucrosomial iron is directed straightly to the lymphatic system and then to the systemic circulation, thus bypassing portal blood stream [28]. This alternative route makes sucrosomial iron able to bypass the ferroportin-dependent outflow from enterocytes, whose expression is negatively influenced by hepcidin levels. This makes sucrosomial iron absorption independent of systemic inflammation, a prominent feature of hemodialysis patients [26]. Also, M cells of the Peyer's plaque can support sucrosomial iron absorption by presenting it to CD68+ macrophages [29].

Considering this particular pharmacokinetic, the oral administration of sucrosomial iron may overcome the concern for oxidative stress as the iron ion is transported directly to the hepatocytes through the sucrosome, thus avoiding the exposure of plasma proteins to the Fenton and Haber–Weiss reactions. Therefore, the rationale of this study is to verify if sucrosomial iron is able to maintain adequate hemoglobin levels and at the same time reduce the levels of oxidative stress biomarkers in comparison with traditional intravenous iron in hemodialysis patients.

Materials and methods

Study design and participants

The study was approved by Humanitas Clinical and Research Center review board before initiation and carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). Thirty hemodialyzed patients in treatment with sodium ferric gluconate complex (62.5 mg) have been initially screened. Patients have been enrolled at the Dialysis Center of Istituto Clinico Humanitas, Rozzano, Milan, Italy. An informed consent has been obtained for each participant. Five patients have been excluded because of the necessity of more than one sodium ferric gluconate complex administration per week. Patients enrolled interrupted the therapy with sodium ferric gluconate complex and started to take Sucrosomial iron (Sideral Forte®, Pharmanutra, Italy) for three months at a dosage of 30 mg for every dialysis session to ensure compliance (total 90 mg per week). A washout period was not considered because it may have determined a significant increase in subsequent iron requirement and a significant decrease of oxidative stress biomarkers before starting sucrosomial iron. A report for eventual side effects has been fulfilled monthly. The only relevant severe adverse event consisted of one patient who developed gut

ischemia that was eventually fatal before completion of the study and was deemed not to be associated with the research drug. The final population thus included 24 patients who completed the 3-month study and have been included in the per-protocol analysis.

Statistical analysis

Statistical analysis has been carried out with the software Stata (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC.). The sample size was determined with a power of 80% and a type 1 error of 5%, considering clinically relevant a reduction of di-Tyr from 0.20 to 0.18 AU, with a common standard deviation of 0.04 AU, according to our previous experience in this setting [41]. Descriptive analysis has been reported as mean ± standard deviation or median and interquartile range, depending on data distribution. The statistical tests used were Mixed Effect REML Regression for time-dependent variations in the studied clinical and research biomarkers, and Student's *t* and Mann–Whitney *U* tests for baseline differences, as appropriated according to data distribution. A *p* value less than 0.05 has been considered significative.

Blood samples

Blood samples have been obtained at the beginning of the study and every month for 3 months. Blood has been taken from the arteriovenous fistula or from a central venous catheter before starting dialysis (after 1 day inter-dialytic interval). No fasting was requested before blood samples were taken. Blood samples for biochemical parameters have been obtained and analyzed at the Clinical Analysis Laboratory of Istituto Clinico Humanitas according to standardized procedures.

Oxidative stress biomarkers

For the measurement of oxidative stress biomarkers, 10 mL of venous blood has been taken with ethylenediaminetetraacetic acid (EDTA) as anticoagulant. Within one hour, the blood samples have been centrifuged at 3000 RPM for 15 min, obtaining plasma aliquots. Aliquots have been stored at − 80 °C until the execution of the different analyses.

Detection of plasma protein carbonylation by SDS-PAGE and western blot

Plasma proteins were fractionated on 12.5% (w/v) reducing SDS-PAGE gels and electro-blotted onto a polyvinylidene difluoride (PVDF) membrane (Biorad). Protein carbonylation was detected, after derivatization with 2,4-dinitrophenylhydrazine (DNPH) (Sigma-Aldrich), with anti-DNP antibodies (dilution 1:20,000) specific for the DNP hydrazone-carbonyl adduct by Western blot immunoassay as previously reported [30]. Immunoreactive protein bands were visualized by enhanced chemiluminescence (ECL) using ChemiDoc Imaging System (Biorad). Protein bands on PVDF membranes were then visualized by washing the blots extensively in PBS, stained with Amido Black and acquired using ChemiDoc Imaging System (Biorad).

Determination of plasma protein carbonyls by enzyme-linked immunosorbent assay (ELISA)

Plasma PCOs were quantified using the ELISA kit manufactured by Enzo Life Sciences (ALX-850–312-KI01). Carbonylated protein standard (40 mg/ml containing 0–0.12–0.22–0.42–0.7–0.9 nmol carbonyls/mg protein) and human plasma samples (60–75 mg/ml) were diluted 1:40 in DNPH solution and incubated 45 min to allow PCO derivatization. A 1:200 dilution in ELISA buffer was then performed before adding 200 µL (1–2 µg of protein) in each ELISA plate well. We incubated ELISA plate overnight at 4 °C to allow protein binding. ELISA assay was performed according to the manufacturer's instructions. Absorbance of plate wells was read at 450 nm using the EnSight™ multimode plate reader (Perkin Elmer). In all the performed assays, calibration line showed an R2 close to 0.99. We then calculated carbonyl content of samples using the regression factors (intercept with the y-axis and line slope) obtained from standard curve.

Determination of plasma AOPPs and protein-bound di-Tyr

Eluates were monitored both at 340 nm for measuring advanced oxidation protein products (AOPPs) absorbance and at 215 nm for measuring the absorbance of peptide bonds using HPLC detector 332 from Kontron Instrument SpA (Milan, Italy). The ratio A_{340}/A_{215} was calculated for each plasma sample. For protein-bound di-tyrosines (di-Tyr) determination, eluates were monitored both at 215 nm for measuring absorbance of peptide bonds and at 415 nm emission with 325 nm excitation using Kontron SFM-25 Spectrofluorimeter equipped with 150 W Xenon N-800-LO, ozone-free, lamp. The ratio between fluorescence and absorbance (IF415nm em/A215nm) was calculated for each sample.

Total plasma thiol determination with 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB)

The free thiol concentration of plasma samples was quantified by the Ellman assay measuring the increase in absorbance at 412 nm caused by the released TNB anion upon reaction of thiols with DTNB (Sigma-Aldrich) and using a molar absorption coefficient of 14.15 mM^{-1} cm^{-1}. In detail, 50 ml

of plasma was diluted with 900 ml of 50 mM potassium phosphate buffer (PBS), pH 7.4, mixed with 50 µl of 3 mM DTNB prepared in PBS and incubated for 15 min at 25 °C. A parallel sample was assembled mixing 950 ml of PBS with 50 µl of plasma for each sample to subtract the intrinsic absorbance of plasma at 412 nm. All measurements were performed in triplicate and the mean intrinsic absorbance was subtracted from the mean absorbance of TNB release. The molar concentration of thiols was calculated from the molar absorbance of the TNB anion.

Determination of plasma protein thiols by means of biotin-maleimide and SDS-PAGE

Biotin-maleimide stock solution was prepared at 40 mM in DMSO (Sigma-Aldrich) and stored at − 20 °C. Plasma protein samples were diluted to a final concentration of 1 mg/mL in 50 mM PBS, pH 7.4, containing 15 µM biotin-maleimide (Sigma-Aldrich). Protein thiol labeling was performed for 1 h at room temperature. After labeling, protein samples were mixed with an equal volume of 2 × reducing Laemmli sample buffer, boiled for 5 min at 90 °C and analyzed by SDS-PAGE using 10% (w/v) Tris–HCl polyacrylamide gels.

After electrophoretic run, proteins were transferred to PVDF membrane and biotin tag revealed with streptavidin-HRP. Briefly, PVDF membranes were washed with PBST [10 mM Na-phosphate, pH 7.2, 0.9% (w/v) NaCl, 0.1% (v/v) Tween 20] and blocked for 1 h in 5% (w/v) non-fat dry milk in PBST. After washing three times with PBST for 5 min each, biotin tag was probed by 2-h incubation with 5% non-fat dry milk/PBST containing 1:5000 dilution of streptavidin-HRP (Cytiva RPN1231). After three washes with PBST, biotinylated proteins were visualized by ECL detection using ChemiDoc Imaging System (Biorad) (Fig. 1). Protein bands on PVDF membranes were visualized by washing the blots extensively in PBS and then stained with Amido Black. ECL signals and protein bands intensity were quantified using Image Lab Software version 5.2.1 (Biorad).

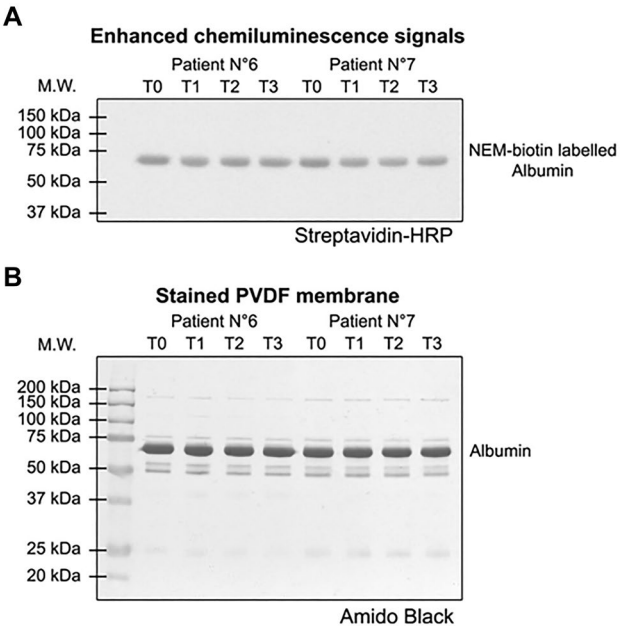

Fig. 1 Determination of plasma protein thiols by means of biotin-maleimide and SDS-PAGE. **A** Digital image of biotinylated proteins after visualization by ECL detection mode (ChemiDoc Imaging System). **B** Protein bands on PVDF membrane after staining with Amido Black and acquisition by colorimetric mode (ChemiDoc Imaging System). Protein molecular weight (M.W.) is shown on the left

Results

From the initial population of 30 patients screened, 5 were excluded for high requirements of intravenous iron to maintain hemoglobin level (> 1 intravenous injection a week) and 1 died before completing the study, so the final per-protocol population consisted of 24 patients (Fig. 2). Patients' characteristics are listed in Table 1. Median age was 75 years [67.5–83.5], with 60% of study participants being males with 2561 days (IQR 2162–9940) of dialysis vintage. Diabetes and cardiovascular disease were present in 36% and 56% of the population, respectively, and 72% of patients were dialyzing with an arteriovenous fistula.

In Table 2, the main anemia-related biochemical parameters across the study are shown. Hemoglobin values remained stable during the three months period of treatment with Sucrosomial iron. The mean changed from 11.2 ± 1.0 g/dL at baseline to 11.0 ± 1.0 g/dL at the conclusion of the study ($p = 0.45$). Also, the values of hematocrit and mean corpuscular volume remained stable, passing, respectively, from 34.7 ± 2.89% to 34.14 ± 3.08% ($p = 0.46$) and from 92.51 ± 8.62 fL to 92.15 ± 8.38 fL ($p = 0.53$). ESA dosage remained stable (from 8000 UI/week (4000–12,000 UI) to 10,000 UI/week (5000–14,000 UI), $p = 0.24$). Ferritin values were lower at the end of the study, resulting 97.19 ± 97.25 ng/mL from 225.50 ± 167.00 ng/mL ($p < 0.01$). Also, transferrin saturation and serum iron were lower at the end of the study (respectively, 16.8 ± 7.3% from 30.0 ± 9.9%, $p < 0.01$; 45.91 ± 20.31 mcg/dL from 70.37 ± 24.91 mcg/dL, $p < 0.01$). Transferrin increased from 1.66 ± 0.32 g/L to 2.05 ± 0.43 g/L ($p < 0.01$). In Fig. 3, other biochemical parameters related to inflammation and nutrition are shown. C-reactive protein reduced non-significantly from 1.46 ± 3.59 mg/dL to 0.95 ± 1.51 mg/dL (p = 0.42). White blood cells reduced from 6.4 ± 1.82 × 10^3/mmc to 5.84 ± 1.38 × 10^3/mmc (p = 0.02).

Fig. 2 Study flow-chart

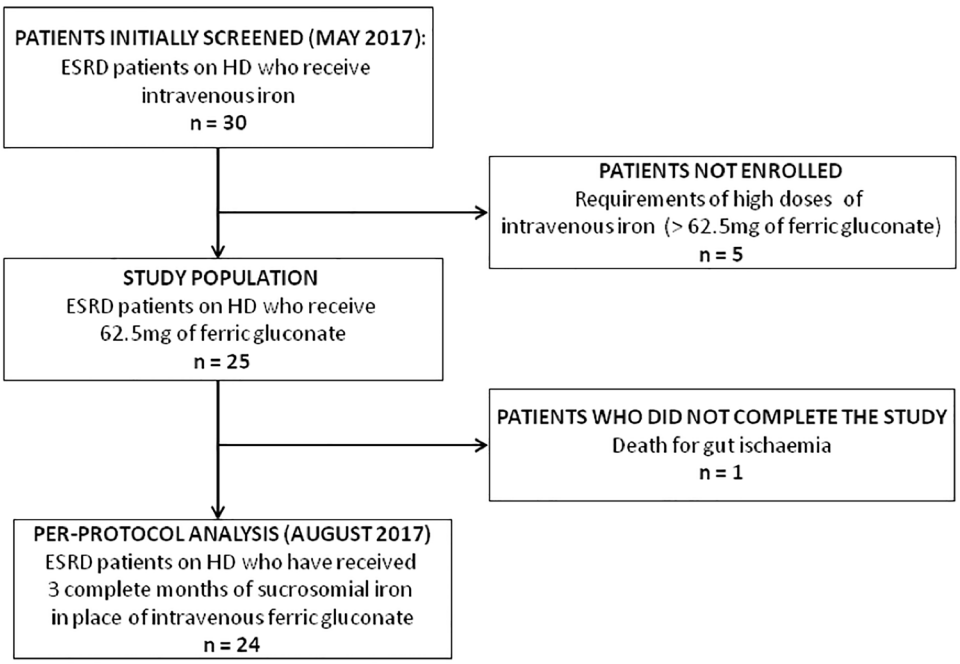

Table 1 Baseline characteristics of study participants

Age	75 [67.5–83.5]
Gender (% men)	15/25 (60%)
Dialysis vintage (months)	2561 [2162–9940]
BMI	25.9 ± 6.9
Diabetes (% of patients affected)	9/25 (36%)
Type of dialysis (HD/HDF)	19/6
Cardiovascular disease (% of patients affected)	14/25 (56%)
Vascular access for dialysis (% of arteriovenous fistula)	18/25 (72%)
Malnutrition-Inflammation Score [24]	4.5 [4–8.5]
ESAs dosage (UI/week)	9440 ± 7269

Values are expressed as mean ± standard deviation or frequency, median with inter-quartile range or frequency, where appropriated

In Table 3, the results of oxidative stress biomarkers analysis are shown. Protein carbonyl groups reduced from 0.13 ± 0.14 nmol/mg to 0.10 ± 0.10 nmol/mg ($p = 0.088$). There was also a non-significant reduction in protein-bound di-Tyr and AOPPs (respectively, 0.19 ± 0.04 AU, 0.18 ± 0.04 AU, $p = 0.112$ and 0.91 ± 0.23 AU, 0.88 ± 0.18 AU ($p = 0.165$)). Protein thiol level did not change from the beginning to the end of the study, total plasma thiol level increased from 4.02 ± 0.99 pmol/μg prot to 4.27 ± 1.79 pmol/μg prot ($p = 0.714$). One patient, as mentioned above, did not complete the 3-month study period having died for gut ischemia. No other adverse events were reported in the same time frame.

Table 2 Hemoglobin, ferritin, TIBC, serum iron and transferrin saturation levels during the three months of therapy with sucrosomial iron

	T0	T1	T2	T3	*P*	Δ 0–3 (95% CI)
Hemoglobin (g/dL)	11.2 ± 1.0	11.1 ± 1.0	11.4 ± 1.0	11.0 ± 1.0	0.681	− 0.175 (− 0.652–0.302)
Ferritin (ng/mL)	225 ± 166	188 ± 147	166 ± 155	97 ± 97	< 0.001	− 128 (− 176 to − 80)
Transferrin TIBC (g/L)	1.66 ± 0.32	1.75 ± 0.42	1.84 ± 0.51	2.05 ± 0.43	< 0.001	0.38 (0.29–0.48)
Serum iron (mcg/dL)	70 ± 24	58 ± 24	51 ± 17	46 ± 20	< 0.001	− 24.5 (− 36.2 to − 12.7)
Transferrin saturation (%)	30.0 ± 9.9	22.7 ± 8.2	20.1 ± 8.3	16.8 ± 7.3	< 0.001	− 13.2 (− 18.25–8.15)
Epo weekly dosage (median UI)	8000 (IQR 4000–12,000)	8000 (IQR 4000–12,000)	9000 (IQR 4000–13,000)	10,000 (IQR 5000–14,000)	0.24	2000 (2000–4000)

Statistical analysis performed with mixed-Effects REML Regression

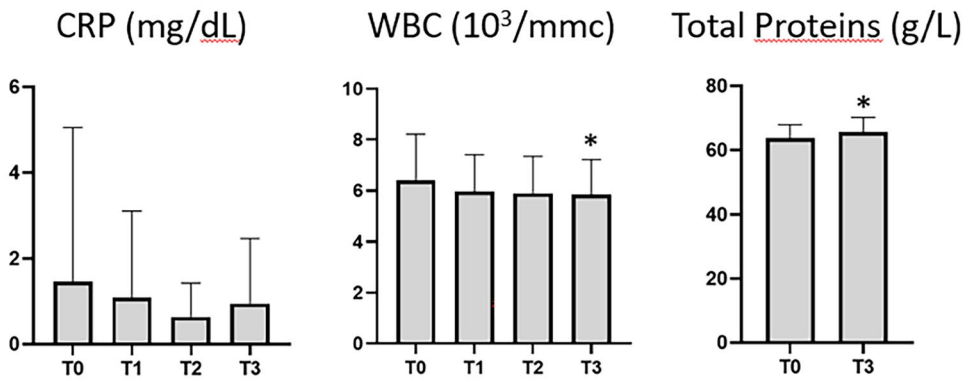

Fig. 3 CRP levels, white blood cells count and total proteins during the three months of therapy with sucrosomial iron. Statistical significance is highlighted by an asterisk (*)

Table 3 Mixed-Effects REML Regression of plasma oxidative stress biomarkers

	T0	T1	T2	T3	*P*
Protein carbonyl groups (nmol/mg)	0.13 ± 0.14	0.13 ± 0.16	0.11 ± 0.11	0.10 ± 0.10	0.088
Di-tyrosine (AU)	0.19 ± 0.04	0.19 ± 0.05	0.18 ± 0.04	0.18 ± 0.04	0.112
AOPPs (AU)	0.91 ± 0.23	0.95 ± 0.31	0.90 ± 0.24	0.88 ± 0.18	0.165
Protein thiols (AU)	0.04 ± 0.01	0.03 + 0.02	0.04 ± 0.02	0.04 ± 0.01	0.268
Total thiols (pmol/μg prot)	4.02 ± 0.99	4.53 ± 1.47	3.97 ± 0.91	4.27 ± 1.79	0.714

Discussion

The presence of anemia in dialysis patients is frequent and iron deficiency is the most common reversible cause. The chronic blood losses and impaired intestinal absorption secondary to elevated hepcidin concentrations are responsible for the loss of 1–2 g of iron every year in dialysis patients [31]. Elevated hepcidin levels, caused by the chronic inflammatory status, determine a down-regulation of ferroportin, which is a carrier protein that regulates the transport of iron from enterocytes' cytoplasm to the bloodstream. The consequence of this pathway is a reduction of iron absorption in dialysis patients [32, 33].

Hence, intravenous iron administration has become the preferred route of administration in this population and its superiority to oral one has been widely demonstrated [5–7]. According to KDIGO guidelines, the most used approach is to treat dialysis patients with intravenous iron when transferrin saturation is less than 20% and ferritin less than 200 ng/mL, independently from hemoglobin. If hemoglobin is less than 10.5 g/dL or the patient is in treatment with ESAs, the suggested cut-off is a transferrin saturation of 30% and a ferritin level of 500 ng/mL [8]. In recent years, new sucrosomial iron has emerged as a possible alternative to intravenous iron in dialysis patients, since its peculiar absorption is independent from the ferroportin/hepcidin axis and thus is not influenced by the micro-inflammatory status [28, 29]. A clinical study on CKD patients, not on dialysis, has demonstrated that sucrosomial iron is effective in maintaining Hb values, even though with lower iron in a 3-month period [34]. To our knowledge, no studies have investigated the safety and effectiveness of sucrosomial iron in hemodialysis patients so far. We analyzed in our study the effect on hemoglobin values and iron metabolism parameters shifting from sodium ferric gluconate complex to Sucrosomial® iron. Sucrosomial iron has been administered at a dosage of 90 mg per week. Previously, patients were taking sodium ferric gluconate complex at a dosage of 62.5 mg per week. During the study, we did not report any adverse events attributable to sucrosomial iron, which proved to be well tolerated. Hemoglobin levels remained stable during the study and the required ESA dosage did not change significantly. However, we observed a significant reduction of ferritin and transferrin saturation. The stability of hemoglobin levels after three months of therapy with sucrosomial iron suggests that this iron formulation may be an alternative to intravenous iron in dialysis patients and is safe. However, it is likely that the dosage we used to ensure patient's compliance (30 mg every hemodialysis [HD] session, 90 mg/week) is insufficient to maintain iron stores in the long-term as suggested by the main guidelines. In fact, patients showed a tendency to develop a functional iron deficiency that, if uncorrected, will likely bring to a reduction of hemoglobin levels or an increase in ESAs dosage eventually. In the future studies, it would be likely to determine if sucrosomial iron, at a greater dosage (ideally 30 mg daily, 210 mg a week), is able to maintain an adequate iron storage in dialysis patients at long term (at least, 6–12 months of follow-up).

A strong motivation to evaluate this approach in future studies is the possibility to improve the oxidative status of hemodialysis patients. Oxidative stress, together with the micro-inflammatory status, represents one of the causes of cardiovascular and metabolic complications of patients on hemodialysis [35]. Different studies have demonstrated that intravenous iron administration is associated with increased oxidative stress biomarkers in dialysis patients [15–20]. This may determine a vicious circle, in which oxidative stress further reduces intestinal iron absorption and determines an increase in intravenous iron demand. Increased oxidative stress seems to be caused by the generation of unbound free iron, a potential trigger of Haber–Weiss and Fenton reactions, after intravenous iron infusion [6]. Under normal conditions, there are different mechanisms to counteract the appearance of free iron and the production of reactive oxygen species secondary to iron metabolism, such as hydroxyl radicals, hydrogen peroxide and superoxide [13]. The rapid infusion of intravenous iron may overtake these protective mechanisms [22]. Moreover, Lim et al. observed higher levels of oxidative stress biomarkers in patients with higher ferritin values (> 600 ng/mL) [15]. These data suggested that intravenous iron may be a potential target in the perspective of reducing oxidative stress in dialysis patients. However, in a metanalysis by Hougen et al., a correlation between higher dosages of intravenous iron and mortality, infections and cardiovascular events has not been observed [36]. On the contrary, in the recent PIVOTAL study, higher dosages of intravenous iron were associated with a reduction in cardiovascular events and mortality rate in dialysis patients [37]. Karaboyas et al. performed an analysis of DOPPS data observing that there is a diffuse increase in mean ferritin values, in particular in the United States and Europe]. This increase in ferritin values is also associated with an increase in mortality [9]. Therefore, it is unclear if higher dosages of intravenous iron or higher levels of ferritin are truly associated with an increased risk of cardiovascular events and mortality mediated by an increased oxidative stress. The scenario is further complicated by the fact that ferritin may also be a marker of inflammation [38]. In this setting, we chose to investigate the possible beneficial effect of sucrosomial iron on oxidative stress. In fact, the peculiar intestinal absorption of sucrosomial iron prevents the generation of labile iron available for redox reactions [39]. With this aim we evaluated, before and after the three months of therapy with sucrosomial iron, the levels of protein carbonyl groups, protein-bound di-Tyr, AOPPs and thiol groups, analyzing in this way different oxidative pathways [40–42]. Protein carbonyl groups are the most general and the most commonly used biomarkers of severe oxidative protein damage. They may result from direct oxidation of lysine, arginine, proline, and threonine residues and interaction with reactive carbonyl species derived from carbohydrate and lipid oxidation or non-oxidative reactions with dicarbonyl compounds. Protein-bound di-Tyr is chemically stable and easily detectable product of tyrosine oxidation in response to oxidative stress induced by both non-enzymatic and peroxidase-catalyzed mechanisms and is a biomarker of irreversible protein oxidation. AOPPs are a heterogeneous group of di-Tyr, pentosidine and carbonyl-containing protein products generated in plasma proteins by both myeloperoxidase-dependent (e.g., in ESRD patients) and MPO-independent (e.g., in the pre-dialysis phase of CKD) mechanisms during oxidative/chlorine stress. As their molecular composition has not yet been precisely defined, AOPPs are considered a generic biomarker of protein oxidation. Alteration in the redox status of plasma thiols can be a diagnostic indicator of different pathological states, including chronic renal failure. Actually, total plasma thiols are mainly constituted by protein thiols, whose concentration is mostly due to the single free thiol at Cys34 of albumin. The remaining part of the plasma thiols is made up of low molecular weight thiols, such as cysteine and homocysteine. We did not observe a statistically significant change of these oxidative stress biomarker values after the switch from sodium ferric gluconate complex to sucrosomial iron. We just observed a not significant reduction in the levels of protein carbonyl groups, protein-bound di-Tyr, and AOPPs. We also observed a slight reduction in CRP values, which may suggest an improvement in inflammatory status, while white blood cells count significantly decreased. These results are not sufficient to prove that the shift to sucrosomial iron is able to reduce oxidative stress biomarkers. This is possibly a consequence of the limitations of this study, which are the study design (observational study without a control group), the duration of the study and the limited size. Another limitation was that baseline intravenous iron therapy (62.5 mg) was not likely associated with severe functional iron deficiency, which is quite common in the HD population. Considering also the mixed results present in the literature [15–20], more studies with longer-term follow-up and an optimal dosage (30 mg daily) are needed to fill this gap in the knowledge.

Conclusion

Our study demonstrates that a therapy with sucrosomial iron in hemodialysis patients is safe and can maintain stable hemoglobin levels in a three-month period. However, the reduction of ferritin and transferrin saturation suggests that a weekly dosage of 90 mg is not sufficient in hemodialysis patients in the long time. This therapy also determined a slight reduction of different oxidative stress biomarkers; however, this result is not significant and must be confirmed in controlled studies with a greater size and a longer duration. Although this study does not give a definitive answer

on the possible advantage of oral iron formulation in dialysis patients, it may represent a starting point for further studies to investigate the complex correlation between iron supplementation, oxidative stress, inflammation, cardiovascular disease, and mortality.

Author contributions FR was involved in data collection and analysis and writing of the manuscript, GC, EA, LL, AM and IDD were involved in the research protocol, analysis of oxidative stress biomarkers and writing of the manuscript, SF and CA were involved in the research protocol and patients recruitment, DC conceived the study and was involved in the research protocol, data analysis and writing of the manuscript.

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Data availability Data are available under reasonable circumstances by direct contact with the corresponding author.

Code availability Not applicable.

Declarations

Conflicts of interest DC has received speaker fees from Pharmanutra. All the other Authors reported no conflict of interest.

Ethical approval The study was approved by Humanitas Clinical and Research Center review board before initiation and carried out according to the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Consent to participate A signed informed consent has been obtained for each participant.

Consent for publication All the authors agree on the publication of the manuscript.

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