

APPENDIX

Appendix A

List of chemicals and reagents used in this study

Chemicals	Sources
Acetonitrile	Fisher Scientific
Ammonium ferric citrate	BDH chemical, England
Ammonium ferrous sulfate	E. Merck, Germany
Bathophrenanthroline	Fluka, Switzerland
Butylated Hydroxytoluene	Sigma-Aldrich, USA
Citric acid	BDH chemical, England
Curcumin	Sigma-Aldrich, USA
Cyamethemoglobin	Sigma-Aldrich, USA
Diacetyl monoxime	Fluka, Switzerland
2', 7'-dichlorofluorescein diacetate	Sigma-Aldrich, USA
1,2-dimethyl-3-hydroxypyridin-4-one (L1)	GPO of Thailand
Ferric chloride	Sigma-Aldrich, USA
Ferric nitrate	Asia Pacific chemical
HAEMO-A	AEONMED, Thailand
HAEMO-B	AEONMED, Thailand
Hydrogen peroxide	Sigma-Aldrich, USA
Thiolsemicarbazide	Sigma-Aldrich, USA
Magnesium carbonate light	UNILAB, Australia
Methanol	E. Merck, Germany
Nirilotriacetic acid disodium salt	Sigma-Aldrich, USA
Nirilotriacetic acid trisodium salt	Fluka, Switzerland
3-[N-Morpholino]propanesulfonic acid (MOPS)	Sigma-Aldrich, USA
Phosphoric acid	E. Merck, Germany
Potassium cyanide	Fluka, Switzerland
Potassium ferric cyanide	Fluka, Switzerland

1-propyl-2-methyl-3-hydroxypyridin-4-one (CP22)	GPO of Thailand
Sodium carbonate	E. Merck, Germany
Sodium chloride	E. Merck, Germany
Sulfuric acid	Labscan
2-Thiobarbituric acid	Sigma-Aldrich, USA
Thioglycolic acid	E. Merck, Germany
TMP	Sigma-Aldrich, USA
Trichloroacetic acid	E. Merck, Germany

Appendix B**List of instruments used in this study**

Instrument	Company
Automatic pipette	GIBCO
Balance	Precisa
Carbondioxide incubator	SHEL LAB
Centrifuge	Hettich
Dialysis membrane	Sigma-Aldrich
Dionized water machine	Barnstead
Distilled water machine	Hamilton
ELISA plate reader	BIOTEK Instrument
Flow cytometer	Becton-Dickinson
High Performance Liquid Chromatography	LDC Analytical
pH meter	Eutech Cybernetics
Polysulfone membrane filter	Pall
Refrigerator	SHARP
Serological pipette	Pyrex
UV visible spectrophotometer	Shimadzu
Vortex	Scientific industries
Water bath	Labline

Appendix C

Preparation of some reagents and buffers

1. Acid solution

Add conc. H_2SO_4 100 ml into distilled water 300 ml, leave it cool. Dissolved thiosemicarbazid 0.5 g and cadmium sulfate 2 g in to H_2SO_4 solution, then added ferric chloride reagent 1 ml. Adjust volume with distilled water to 1,000 ml.

2. Oxime solution

Dissolved diacetyl monoxime 1.25 g in distilled water 1,000 ml.

3. Drakkin's reagent

NaHCO_3	1	g
KCN	0.05	g
$\text{K}_3\text{Fe}[\text{CN}]_6$	0.2	g

Dissolved in distilled water, adjust volume to 1,000 ml. Store in dark brown bottle.

4. Protein precipitant solution

Placed in a 1-L volumetric flask 33.3 ml of thioglycolic acid, 98.4 ml of trichloroacetic acid solution and 400 ml of deionised water. Swirled to dissolve. Slowly added 2 mol of HCl. Added deionised to volume. Stored in a dark brown bottle.

5. Chromogen solution

Placed in a 1-L volumetric flask 250 mg of disodium bathophenanthroline disulfonic acid and 84.04 g of sodium acetate. Added deionised to volume.

6. Ammonium ferric citrate

Placed 400 mg ammonium ferric citrate in a 1-L volumetric flask and added 950 ml distilled water. Added a few crystals of citric acid and shake to dissolve. Adjust pH to 7.0 with dilute NH_4OH and dilute to volume with deionised water. Stored at 4 °C and avoid prolonged exposure to light. Solution should be alight yellow-green and free of rust-colored precipitate.

7. MOPS buffer solution

Dissolved 20.94 of 3-[N-Morpholino]propanesulfonic acid (MOPS) in 900 ml of deionised water, then adjust to pH 7.0 with 10 N NaOH, and to a final volume of 1 liter with deionised water.

8. Curcumin solution

Curcuminoid containing curcumin, demethoxycurcumin and bisdemethoxycurcumin was dissolved in 0.1M MOPS buffer (pH 7.0).

9. Deferiprone solution

Dissolved deferiprone (L1) in 0.1 M MOPS buffer (pH 7.0) solution to obtain a concentration of 1 mM.

10. NTA solution

N,N-bis(carboxymethylglycine) disodium salt 94.04 g

N,N-bis(carboxymethylglycine) trisodium salt 102.84 g

Dissolved separately in 500 ml of deionised water. These two NTA solutions (800 mM each) were mixed until the final pH of 800 mM NTA solution came to 7.0.

10.1) 80 mM NTA solution

Prepared by mixing 100 ml of 800 mM NTA solution with 900 ml of deionised water and used as a diluting solution to prepare several iron standard solutions.

10.2) 10 mM NTA solution

Prepared by mixing 10 ml of 800 mM NTA solution with 790 ml of deionised water.

11. Iron solution

Ferric nitrate in 1 N HNO₃ solution (1 ppm. equivalent to 18.76 mM) was used as a stock chemical iron solution and diluted for preparing ferric nitrate at required concentrations.

Ferric citrate solution was prepared by adding ferric nitrate solution into the citric acid solution (1mM, pH 5.5) to reach required concentrations.

For the HPLC-based NTBI measurement, standard iron solution was prepared as follow:

80 μ M iron solution was prepared by adding 0.21 ml of stock iron solution (18.76 mM) to 50 ml of 800 mM NTA solution and allowed to stand at room temperature for 1 hour. Then 10 ml of 80 μ M Fe present in 800 mM NTA solution was diluted with 40 ml of deionised water to obtain a 16 μ M Fe solution which was the highest concentration of working iron solution concentration. Other standard iron solution at final concentrations of 0, 2, 4 and 8 μ M Fe was prepared from 16 μ M Fe by diluting with 80 mM NTA.

solution. Finally, all standard iron solution were made 0.5 ml aliquots in propylene microtubes and kept frozen at -20°C .

12. Mobile-phase solvent for NTBI analysis

Dissolved 0.625 g of 3-hydroxy-1-propyl-2-methylpyridin-4-one (CP22) in 750 ml of deionised water and 50 ml of 0.1 M MOPS solution, then adjusted pH to 7.0 with 10 N NaOH. The mixture was ultrafiltered and degassed on cellulose acetate membrane (pore size $0.45\ \mu\text{m}$, 43 mm in diameters) equipped on Millipore Ultrafiltration apparatus. Added 190 ml of HPLC-grade acetonitrile and finally adjusted the final volume of 1 liter with deionised water. This mobile-phase solvent is freshly prepared or degassed before use.

13. DCFH-DA solution

Dissolved DCFH-DA 0.01 g in methanol 1 ml. Stored in dark container, -4°C .

14. Dialysate solution

HAEMO-A; Content per Litre

Sodium chloride	210.7	g
Potassium chloride	5.222	g
Calcium chloride $2\text{H}_2\text{O}$	9.0	g
Magnesium chloride $6\text{H}_2\text{O}$	3.558	g
Glacial acetic acid	6.31	g
Dextrose monohydrate	77	g

HAEMO-B; Content per Litre

Sodium bicarbonate	84	g
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Mixed HAEMO-A with HAEMO-B in a fixed proportion of 1:1.5. This working dialysate solution was freshly prepared before used.

15. Thiobarbituric Acid solution

Dissolved 0.3 g of thiobarbituric acid (0.6 %, w/v) in 50 ml of deionised water and heated in water bath to 60°C to dissolve.

16. Butylated hydroxytoluene solution.

Dissolved 0.2 g of butylated hydroxytoluene (0.2%, w/v) in 100 ml of ethanol.

Appendix D
Subject history

Table S-1.1 Clinical history of eleven healthy volunteers.

Study population	Gender	Age (Yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
N1	F	24.0	No	No	No	No
N2	M	23.0	No	No	No	No
N3	M	26.0	No	No	No	No
N4	M	26.0	No	No	No	No
N5	M	21.0	No	No	No	No
N6	F	22.0	No	No	NO	No
N7	F	22.0	No	No	No	No
N8	F	22.0	No	No	No	No
N9	F	22.0	No	No	No	No
N10	F	22.0	No	No	No	No
N11	F	23.0	No	No	No	No
Mean±SD	4M, 7F	23±1.7	-	-	-	-

Table S-1.2 Hematological parameters of iron status and oxidative stress in blood samples of eleven healthy volunteers.

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
N1	38	11.7	51	205
N2	52	15.2	40	170
N3	50	15.0	33	165
N4	48	15.5	9	106
N5	35	13.3	3	157
N6	40	11.7	36	154
N7	43	15.4	28	316
N8	44	15.7	53	246
N9	44	15.7	29	361
N10	40	14.2	54	424
N11	40	14.3	11	75
Mean±SD	43.1±5.2	14.3±1.5	30.4±19.7	227.1±119.1

Table S-1.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of eleven healthy volunteers.

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
N1	9.2	31	29.8	-0.44	5.9	8.3
N2	26.4	87.7	30.1	0.83	7.5	10.2
N3	16.6	67.7	24.5	0.39	9.0	8.6
N4	18.9	49.7	38.1	0.83	13.1	17.3
N5	24.5	60.3	40.6	0.24	6.5	11.6
N6	10.9	43.4	25.0	-0.72	7.8	5.1
N7	27.3	56.3	48.5	-2.26	5.3	16.6
N8	23.3	52.3	44.6	-0.8	5.5	16.5
N9	40.0	52.0	76.9	-1.38	6.3	17.2
N10	11.7	51.3	22.7	-2.26	6.4	11.1
N11	23.7	63.7	37.2	-2.99	6.1	9.4
Mean $\pm$ SD	21.1 $\pm$ 8.9	56 $\pm$ 14.4	38 $\pm$ 15.5	-0.78 $\pm$ 1.31	7.2 $\pm$ 2.2	12 $\pm$ 4.2

Table S-2.1 Clinical history of seven end-stage renal disease patients on regular hemodialysis and intermittent blood transfusions (ESRD-T).

Study population	Gender	Age (Yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
ESRD-T1	M	39	95	9	No	Yes
ESRD-T2	M	68	161	2	No	Yes
ESRD-T3	M	65	66	1	No	Yes
ESRD-T4	F	41	95	2	No	Yes
ESRD-T5	M	62	192	1	No	Yes
ESRD-T6	F	65	82	17	No	Yes
ESRD-T7	F	64	152	13	No	Yes
Mean±SD	4M, 3F	58±12	120±47	6.4±6.5	-	-

Table S-2.2 Hematological parameters of iron status and oxidative stress in blood samples of seven end-stage renal disease patients on regular hemodialysis and intermittent blood transfusions (ESRD-T).

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
ESRD-T1	27	8.8	2	98
ESRD-T2	27	6.9	6	192
ESRD-T3	39	11.6	10	211
ESRD-T4	20	5.3	21	163
ESRD-T5	27	7.5	6	238
ESRD-T6	25	6.5	2	150
ESRD-T7	22	8.3	109.14	186.74
Mean±SD	26.7±6.1	7.8±2	22.3±38.8	177±45.4

Table S-2.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of seven end-stage renal disease patients on regular hemodialysis and intermittent blood transfusions (ESRD-T).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-T1	12.1	25.9	47.0	3.37	5.8	41.0
ESRD-T2	8.7	12.2	72.0	9.38	16.9	34.6
ESRD-T3	17.0	38.0	45.0	2.27	4.5	23.7
ESRD-T4	23.0	34.1	68.0	11.51	11.4	26.2
ESRD-T5	11.7	26.6	44.0	2.27	1.5	25.2
ESRD-T6	21.3	32.3	154.7	7.95	15.4	18.7
ESRD-T7	27.3	38.7	70.7	7.4	4.9	46.8
Mean $\pm$ SD	17.3 $\pm$ 6.8	29.7 $\pm$ 9.2	71.6 $\pm$ 38.7	6.3 $\pm$ 3.7	8.6 $\pm$ 6	31 $\pm$ 10

Table S-3.1 Clinical study of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E).

Study population	Gender	Age (yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
ESRD-E1	M	71	10	No	Yes	Yes
ESRD-E2	M	76	231	No	Yes	Yes
ESRD-E3	M	NA	195	No	Yes	Yes
ESRD-E4	F	73	167	No	Yes	Yes
ESRD-E5	M	79	22	No	Yes	No
ESRD-E6	F	78	63	No	Yes	No
ESRD-E7	F	65	222	No	Yes	Yes
ESRD-E8	M	71	232	No	Yes	Yes
ESRD-E9	F	82	341	No	Yes	Yes
ESRD-E10	M	74	37	No	Yes	Yes
ESRD-E11	F	70	195	No	Yes	Yes
ESRD-E12	M	69	239	No	Yes	Yes
ESRD-E13	M	69	NA	No	Yes	Yes
ESRD-E14	M	65	363	No	Yes	Yes
ESRD-E15	M	92	258	No	Yes	Yes
ESRD-E16	F	79	174	No	Yes	Yes
ESRD-E17	F	85	39	No	Yes	Yes
ESRD-E18	M	64	342	No	Yes	Yes
ESRD-E19	F	44	77	No	Yes	Yes
ESRD-E20	F	66	261	No	Yes	Yes
ESRD-E21	M	66	239	No	Yes	Yes

Table S-3.1 Clinical study of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Gender	Age (yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
ESRD-E22	M	71	229	No	Yes	Yes
ESRD-E23	F	73	168	No	Yes	Yes
ESRD-E24	M	53	50	No	Yes	Yes
ESRD-E25	F	65	223	No	Yes	Yes
ESRD-E26	M	77	340	No	Yes	Yes
ESRD-E27	F	78	64	No	Yes	No
ESRD-E28	M	82	31	No	Yes	Yes
ESRD-E29	M	79	23	No	Yes	No
ESRD-E30	M	65	364	No	Yes	Yes
ESRD-E31	F	73	90	No	Yes	Yes
ESRD-E32	M	60	215	No	Yes	Yes
ESRD-E33	F	NA	17	No	Yes	NA
ESRD-E34	M	71	23	No	Yes	Yes
ESRD-E35	M	67	177	No	Yes	Yes
ESRD-E36	F	NA	18	No	Yes	NA
ESRD-E37	F	70	396	No	Yes	Yes
ESRD-E38	F	67	153	No	Yes	Yes
ESRD-E39	F	73	170	No	Yes	Yes
ESRD-E40	M	66	33	No	Yes	Yes
ESRD-E41	M	60	161	No	Yes	Yes
ESRD-E42	F	73	119	No	Yes	Yes

Table S-3.1 Clinical study of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Gender	Age (yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
ESRD-E43	F	NA	NA	No	Yes	NA
ESRD-E44	M	66	42	No	Yes	Yes
ESRD-E45	M	NA	42	No	Yes	NA
ESRD-E46	M	74	81	No	Yes	Yes
ESRD-E47	F	NA	NA	No	Yes	NA
ESRD-E48	M	54	350	No	Yes	No
ESRD-E49	F	70	128	No	Yes	Yes
ESRD-E50	F	NA	NA	No	Yes	NA
ESRD-E51	F	53	53	No	Yes	Yes
ESRD-E52	M	64	16	No	Yes	Yes
ESRD-E53	F	NA	NA	No	Yes	No
ESRD-E54	M	65	13	No	Yes	Yes
ESRD-E55	M	73	114	No	Yes	Yes
ESRD-E56	F	70	131	No	Yes	Yes
ESRD-E57	M	73	38	No	Yes	Yes
ESRD-E58	F	NA	80	No	Yes	NA
ESRD-E59	M	54	173	No	Yes	Yes
ESRD-E60	M	85	84	No	Yes	Yes
ESRD-E61	M	66	253	No	Yes	Yes
ESRD-E62	M	72	105	No	Yes	Yes
ESRD-E63	F	73	183	No	Yes	Yes

Table S-3.1 Clinical study of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Gender	Age (yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
ESRD-E64	M	82	45	No	Yes	Yes
ESRD-E65	F	67	8	No	Yes	No
ESRD-E66	F	65	738	No	Yes	Yes
ESRD-E67	F	76	15	No	Yes	Yes
ESRD-E68	F	74	54	No	Yes	Yes
ESRD-E69	M	72	43	No	Yes	Yes
ESRD-E70	F	46	177	No	Yes	Yes
ESRD-E71	M	71	28	No	Yes	Yes
ESRD-E72	M	64	76	No	Yes	Yes
ESRD-E73	M	66	156	No	Yes	Yes
ESRD-E74	M	62	41	No	Yes	Yes
ESRD-E75	F	NA	NA	No	Yes	NA
ESRD-E76	F	72	63	No	Yes	Yes
ESRD-E77	M	68	400	No	Yes	Yes
ESRD-E78	M	66	4	No	Yes	Yes
ESRD-E79	F	44	101	No	Yes	Yes
ESRD-E80	F	63	96	No	Yes	Yes
ESRD-E81	F	NA	51	No	Yes	NA
ESRD-E82	M	83	66	No	Yes	Yes
ESRD-E83	F	18	12	No	Yes	Yes
ESRD-E84	M	NA	NA	No	Yes	NA

Table S-3.1 Clinical study of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Gender	Age (yrs)	Hemodialysis (times)	Blood transfusion (times)	rHuEPO (times)	Iron supplement
ESRD-E85	M	NA	NA	No	Yes	NA
ESRD-E86	M	NA	10	No	Yes	NA
ESRD-E87	F	56	138	No	Yes	Yes
ESRD-E88	M	62	157	No	Yes	Yes
ESRD-E89	M	80	149	No	Yes	Yes
ESRD-E90	F	74	48	No	Yes	Yes
ESRD-E91	F	73	196	No	Yes	Yes
ESRD-E92	M	70	30	No	Yes	Yes
ESRD-E93	M	76	287	No	Yes	Yes
ESRD-E94	M	66	86	No	Yes	Yes
ESRD-E95	F	NA	NA	No	Yes	No
ESRD-E96	F	NA	NA	No	Yes	No
ESRD-E97	M	65	446	No	Yes	Yes
ESRD-E98	M	62	313	No	Yes	Yes
ESRD-E99	F	79	231	No	Yes	Yes
ESRD-E100	F	73	144	No	Yes	Yes
ESRD-E101	F	66	318	No	Yes	Yes
ESRD-E102	F	85	65	No	Yes	Yes
ESRD-E103	M	69	294	No	Yes	Yes
Mean±SD	55M,48F	68±10	150±129	-	-	-

Table S-3.2 Hematological parameters of iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E).

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
ESRD-E1	37	10.8	14.9	425.66
ESRD-E2	25	7.8	27.95	1042.64
ESRD-E3	33	10.0	35.82	100.12
ESRD-E4	31	8.9	29.66	446.59
ESRD-E5	32	9.6	32.94	286.29
ESRD-E6	24	6.6	51.21	1883.21
ESRD-E7	26	6.8	44.54	2112.23
ESRD-E8	31	10.3	54.09	117.02
ESRD-E9	40	10.7	55.02	120.06
ESRD-E10	34	9.4	45.2	118.34
ESRD-E11	30	9.8	43.57	230.74
ESRD-E12	28	8.2	53.77	2530.23
ESRD-E13	38	11.1	21.52	119.28
ESRD-E14	42	11.1	26.72	123.21
ESRD-E15	32	9.0	28.33	164.01
ESRD-E16	25	6.5	28.64	165.24
ESRD-E17	42	13.4	99.09	157.65
ESRD-E18	37	11.2	20.03	244.99
ESRD-E19	33	9.8	19.78	356.78
ESRD-E20	38	10.4	54.97	386.66
ESRD-E21	30	9.1	38.44	35.44

Table S-3.2 Hematological parameters of iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
ESRD-E22	29	8.5	40.26	27.16
ESRD-E23	31	10.3	55.85	104.24
ESRD-E24	25	7.4	36.98	162.63
ESRD-E25	26	6.6	35.32	158.02
ESRD-E26	28	7.4	31.18	226.67
ESRD-E27	24	6.7	32.76	40.85
ESRD-E28	38	11.3	27.17	31.18
ESRD-E29	30	8.8	32.19	170.88
ESRD-E30	40	10.9	41.64	105.31
ESRD-E31	28	8.8	21.85	973.14
ESRD-E32	33	9.6	33.08	1070.29
ESRD-E33	35	11.0	46.95	96.03
ESRD-E34	19	5.7	43.28	255.2
ESRD-E35	36	9.7	41.76	543.62
ESRD-E36	37	11.1	35.68	479.04
ESRD-E37	32	11.8	35.03	894.88
ESRD-E38	32	9.0	31.12	766.87
ESRD-E39	40	9.0	15.36	603.01
ESRD-E40	28	8.8	17.06	650.3
ESRD-E41	34	8.6	20.29	363.52
ESRD-E42	38	11.0	10.9	369.31

Table S-3.2 Hematological parameters of iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
ESRD-E43	32	8.4	23.44	725.91
ESRD-E44	27	8.7	18.27	70.12
ESRD-E45	35	8.5	15.51	1093.55
ESRD-E46	32	9.4	21.04	138.52
ESRD-E47	35	9.2	2.21	30.5
ESRD-E48	42	11.9	21.67	462.86
ESRD-E49	30	8.0	19.06	383.27
ESRD-E50	32	9.9	24.27	694.22
ESRD-E51	33	11.2	22.83	471.66
ESRD-E52	23	6.4	46.75	732.03
ESRD-E53	30	9.1	45.5	528.08
ESRD-E54	30	9.9	49.32	830.31
ESRD-E55	43	12.8	40.65	111.83
ESRD-E56	30	8.6	62.09	208.89
ESRD-E57	34	10.3	42.64	117.88
ESRD-E58	33	9.7	45.34	120.84
ESRD-E59	35	10.4	54.21	155.02
ESRD-E60	28	7.5	165.83	106.8
ESRD-E61	30	9.1	9.81	64.35
ESRD-E62	30	9.2	14.81	181.06
ESRD-E63	31	9.0	11.55	54.48

Table S-3.2 Hematological parameters of iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
ESRD-E64	38	11.3	7.05	139.06
ESRD-E65	37	9.8	13.34	143.34
ESRD-E66	26	6.6	12.63	2865.21
ESRD-E67	23	5.8	6.66	147.95
ESRD-E68	33	9.5	17.81	599.77
ESRD-E69	40	8.9	9.86	282.74
ESRD-E70	26	8.2	3.19	333.23
ESRD-E71	38	11.4	6.55	447.53
ESRD-E72	32	9.1	6.8	348.06
ESRD-E73	26	8.2	2.81	140.02
ESRD-E74	34	9.4	4.26	287.23
ESRD-E75	34	9.0	25.17	156.72
ESRD-E76	30	8.5	15.5	296.51
ESRD-E77	32	9.1	18.39	229.65
ESRD-E78	36	10.1	16.33	182.94
ESRD-E79	31	9.8	6.64	92.96
ESRD-E80	33	9.0	5.76	203.68
ESRD-E81	29	8.1	15.61	326.33
ESRD-E82	30	8.3	11.07	363.06
ESRD-E83	25	6.5	4.96	271.71
ESRD-E84	31	7.6	9.18	311.45

Table S-3.2 Hematological parameters of iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	Hematocrit (%)	Hemoglobin (g/dl)	FI	FI*
ESRD-E85	36	10.4	4.84	150.44
ESRD-E86	25	6.7	4.5	220/81
ESRD-E87	28	7.3	7.52	133.26
ESRD-E88	37	10.1	19.6	168.57
ESRD-E89	35	8.8	11.92	329.98
ESRD-E90	38	12.1	17.9	234.52
ESRD-E91	33	11.2	9.74	289.35
ESRD-E92	27	7.0	13.79	230.23
ESRD-E93	35	9.3	15.17	570.21
ESRD-E94	34	10.6	10.76	451.66
ESRD-E95	31	9.2	11.33	1677.62
ESRD-E96	30	7.9	20.92	1344.48
ESRD-E97	40	14.2	11.4	120.68
ESRD-E98	35	10.3	29.1	374.63
ESRD-E99	45	12.7	16	260.67
ESRD-E100	38	10.7	15.99	148.74
ESRD-E101	30	8.7	3.64	235.31
ESRD-E102	45	13.7	17.36	52.34
ESRD-E103	38	13.5	2.7	195.76
Mean±SD	32.5±5.2	9.4±1.8	25.3±22.2	422.9±510.4

Table S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-E1	12.1	52.0	23.2	0.06	5.1	56.2
ESRD-E2	27.7	22.3	124.2	0.6	5.7	61.9
ESRD-E3	12.1	25.7	47.0	3.06	2.8	74.6
ESRD-E4	15.7	31.0	50.5	3.06	6.4	71.6
ESRD-E5	19.6	31.0	63.3	3.02	3.7	53.2
ESRD-E6	9.1	22.7	40.0	0.6	2.3	37.0
ESRD-E7	8.9	54.3	16.3	2.93	4.5	56.7
ESRD-E8	17.7	53.3	33.3	3.62	4.1	79.4
ESRD-E9	31.5	28.7	109.9	1.9	5.5	74.6
ESRD-E10	10.6	50.7	20.9	0.17	3.9	50.5
ESRD-E11	21.9	21.0	104.2	6.61	12.5	48.3
ESRD-E12	20.4	57.7	35.5	2.51	3.8	99.4
ESRD-E13	17.2	24.7	69.6	2.76	3.3	63.7
ESRD-E14	25.8	32.3	79.9	3.47	4.6	52.9
ESRD-E15	20.4	29.3	69.5	2.73	4.7	60.6
ESRD-E16	12.1	48.0	25.2	2.16	2.0	73.0
ESRD-E17	24.0	26.3	91.0	2.07	4.8	57.0
ESRD-E18	22.8	35.7	64.0	0.6	5.1	63.8
ESRD-E19	21.9	50.0	43.8	4.58	4.8	98.6
ESRD-E20	17.9	25.0	71.7	3.81	3.6	47.6
ESRD-E21	5.8	33.0	17.7	0.19	4.6	27.8

Table S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-E22	5.8	52.7	11.1	3.02	10.4	79.7
ESRD-E23	9.6	31.0	31.0	2.39	7.7	43.7
ESRD-E24	9.2	17.7	52.3	0.19	4.2	48.1
ESRD-E25	13.8	19.7	70.0	1.97	9.7	62.4
ESRD-E26	4.9	35.7	13.8	0.97	10.9	59.7
ESRD-E27	18.7	27.7	67.5	0.92	6.5	28.1
ESRD-E28	11.7	38.7	30.3	1.45	6.7	84.4
ESRD-E29	8.1	24.7	32.9	2.72	5.9	33.2
ESRD-E30	9.6	19.0	50.6	4.6	16.2	31.1
ESRD-E31	11.5	24.7	46.7	1.6	6.7	71.6
ESRD-E32	13.6	27.7	49.1	2.5	13.2	29.7
ESRD-E33	18.9	36.0	52.4	0.92	4.7	45.4
ESRD-E34	11.5	28.0	41.1	2.39	8.4	50.2
ESRD-E35	11.5	28.0	41.1	2.39	8.4	50.2
ESRD-E36	9.6	63.0	15.3	1.41	7.5	66.5
ESRD-E37	13.0	27.7	47.1	0.68	12.6	73.0
ESRD-E38	10.0	30.0	33.3	0.24	5.6	66.5
ESRD-E39	9.1	47.0	19.3	0.24	7.3	53.8
ESRD-E40	26.0	24.0	108.5	2	6.5	54.4
ESRD-E41	15.8	131.0	12.1	-0.35	12.2	26.2

Table S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-E42	8.3	20.0	41.5	-0.35	2.1	37.6
ESRD-E43	11.3	13.6	83.0	4.79	8.0	23.3
ESRD-E44	11.9	27.6	43.1	2.44	2.7	2.7
ESRD-E45	21.7	27.6	78.7	0.09	7.5	85.6
ESRD-E46	21.9	27.0	81.2	2.27	7.9	38.6
ESRD-E47	12.1	27.9	43.3	0.63	7.4	55.1
ESRD-E48	10.8	24.5	43.8	3.19	11.5	41.6
ESRD-E49	38.5	37.0	104.1	-0.05	7.5	39.0
ESRD-E50	15.8	47.6	33.3	-0.35	6.9	33.7
ESRD-E51	10.8	23.0	46.7	-0.79	6.8	46.5
ESRD-E52	12.1	59.4	20.3	0.09	8.1	96.0
ESRD-E53	15.8	25.5	62.3	3.96	4.5	37.1
ESRD-E54	6.6	32.4	20.4	-0.2	6.6	68.3
ESRD-E55	14.3	83.6	17.1	0.68	8.5	35.2
ESRD-E56	7.9	38.2	20.8	1.27	4.4	20.3
ESRD-E57	13.4	25.8	52.0	-1.32	4.9	34.0
ESRD-E58	16.0	76.4	21.0	-0.93	7.3	43.3
ESRD-E59	8.3	27.9	29.8	-0.35	4.4	56.0
ESRD-E60	11.5	45.5	25.3	1.61	6.4	33.5
ESRD-E61	10.2	35.5	28.8	0.97	6.6	30.2

Table S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-E62	14.7	34.2	42.9	0.63	12.6	41.3
ESRD-E63	17.7	116.1	15.2	0.09	26.7	27.0
ESRD-E64	15.5	39.1	39.7	0.39	11.9	63.5
ESRD-E65	14.3	39.1	36.5	3.9	4.7	53.8
ESRD-E66	19.8	31.2	63.4	1.12	5.2	59.7
ESRD-E67	22.8	40.3	56.5	1.61	14.0	41.4
ESRD-E68	15.7	32.7	48.1	1.12	2.0	42.5
ESRD-E69	28.7	60.9	47.2	1.82	2.9	65.7
ESRD-E70	6.8	34.8	19.5	1.03	3.0	41.1
ESRD-E71	9.6	24.2	39.5	0.06	4.4	45.7
ESRD-E72	14.7	26.7	55.1	-0.5	10.7	59.4
ESRD-E73	25.7	24.5	104.9	0.62	3.7	31.7
ESRD-E74	21.7	20.9	103.8	0.89	3.0	35.2
ESRD-E75	16.8	21.5	78.1	1.17	4.6	39.0
ESRD-E76	16.0	45.5	35.1	-0.5	4.4	71.9
ESRD-E77	56.0	43.0	130.0	1.45	5.4	55.4
ESRD-E78	27.0	38.2	70.8	-0.5	8.9	44.8
ESRD-E79	8.1	22	36.8	-0.22	12.9	92.7
ESRD-E80	8.7	34.2	25.5	-0.5	4.9	62.1
ESRD-E81	16.8	28.8	58.5	-0.5	3.2	29.2

Table S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-E82	21.5	26.9	80.0	0.42	5.2	52.7
ESRD-E83	34.9	27.2	128.3	0.05	1.9	44.3
ESRD-E84	11.9	54.4	21.9	-0.37	4.7	46.3
ESRD-E85	12.6	23.1	54.3	0.42	5.5	27.0
ESRD-E86	10.6	18.8	56.7	-0.51	5.4	64.6
ESRD-E87	23.4	33.8	69.2	3.19	3.8	28.1
ESRD-E88	13.6	18.1	75.1	1.43	6.6	22.7
ESRD-E89	13.4	26.6	50.5	3.65	2.8	22.4
ESRD-E90	22.3	38.1	58.6	1.34	5.1	47.6
ESRD-E91	18.1	13.1	137.8	1.33	12.4	55.9
ESRD-E92	21.5	15.0	143.3	1.34	6.3	77.0
ESRD-E93	48.5	30.9	156.8	1.33	9.7	54.4
ESRD-E94	25.1	21.9	114.8	4.95	6.1	59.8
ESRD-E95	5.1	14.1	36.3	7.81	4.5	42.4
ESRD-E96	31.5	30.3	103.9	0.88	6.1	36.8
ESRD-E97	35.3	33.1	106.6	1.34	12.6	28.6
ESRD-E98	13	28	46.4	2.45	18.9	32.7
ESRD-E99	29.3	43.3	194.5	7.95	6.1	42.2
ESRD-E100	25.1	45.6	55.0	5.5	6.1	7.0
ESRD-E101	25.3	30.6	82.7	11.51	5.3	44.4

Table S-3.3 Biochemical parameters of renal function, iron status and oxidative stress in blood samples of one-hundred and three end-stage renal disease patients on regular hemodialysis and rHuEPO injection (ESRD-E) (continued).

Study population	PI (μM)	TIBC (μM)	Transferrin saturation (%)	NTBI (μM)	MDA (μM)	BUN (mg/dl)
ESRD-E102	17.9	44.1	40.6	9.66	17.7	32.1
ESRD-E103	22.6	23.4	96.2	4.34	5.9	73.8
Mean $\pm$ SD	17.07 $\pm$ 8.75	35.1 $\pm$ 18	57.7 $\pm$ 35.5	1.76 $\pm$ 2.2	6.8 $\pm$ 4	50.2 $\pm$ 19.2

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