Haptoglobin 2-2 phenotype is associated with decreased serum iron levels in end-stage renal disease patients resistant to rhEPO therapy

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Haptoglobin (Hp) is an acute-phase protein that binds, in an equimolar ratio with high affinity, to haemoglobin released during the intravascular lysis of erythrocytes.[1] This intravascular lysis accounts for approximately 10–20% of normal erythrocyte destruction. The primary function of Hp is to bind this free haemoglobin, which prevents renal excretion of iron and protects blood vessels from the oxidative effects of free haemoglobin.[2]

Haptoglobin is polymorphic, with two co-dominant alleles, Hp1 and Hp2, and three major phenotypes, Hp 1-1, Hp 2-1 and Hp 2-2. The allele Hp1 is encoded by five exons and the Hp2 allele by seven exons. The latter appears to be generated by an intragenic duplication from the first allele. This leads to structural differences and subunit arrangement of Hp phenotypes.[3] This phenotypic heterogeneity is demonstrated by the different sizes of molecule; Hp1-1 is a small molecule of 86 kDa, whereas Hp1-2 is 86–300 kDa, and Hp2-2 forms large macromolecular complexes of 170–1000 kDa.[4] The protein encoded by the Hp2 allelle is a less-potent antioxidant compared with the protein encoded by the Hp1 allele. Recently, it has been demonstrated that individuals with diabetes mellitus presenting with the Hp2-2 phenotype show significantly higher risk of cardiovascular disease.[5,6]

Anaemia is a common complication in haemodialysis (HD) patients, mainly due to a reduction in the kidney production of erythropoietin (EPO). The introduction of recombinant human EPO (rhEPO) therapy has led to a significant reduction in anaemia. However, some HD patients show a marked resistance to rhEPO therapy, which has been associated mainly with functional iron deficiency and with enhanced inflammation.[7]

Functional iron deficiency is associated with a reduction in iron absorption and with iron retention in monocytes/macrophages. The Hp2 allele favours endocytosis of haemoglobin-Hp complexes by monocytes/macrophages, leading to enhanced iron storage and retention as compared to the Hp1 allele.[4]

In the present study the authors hypothesise that the Hp2 allele could influence the iron status of HD patients, particularly those with enhanced inflammation who are usually non-responders to rhEPO therapy.

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The cross-sectional study evaluated 253 HD patients (141 males, 112 females; mean age [±SD]: 65.20 [14.64] years). The HD patients included 201 responders and 52 non-responders to rhEPO therapy. Classification of the patients, as responders or non-responders, was performed in accordance with the European Best Practice Guidelines,8 which define resistance to rhEPO as a failure to achieve target haemoglobin levels (11–12 g/dL) with maintained doses of rhEPO higher than 300 iu/Kg/week of epoetin or 1.5 µg/Kg/week of darbopoietin-α. Patients with autoimmune disease, malignancy, haematological disorders, and acute or chronic infection were excluded. Intravenous iron supplementation was based on the European Best Practice Guidelines for the management of anaemia in patients with HD.[9] All participants gave written informed consent to participate in the study, which was previously approved by the Ethics Committee.

Haematological data was accessed using an automatic blood cell counter (Sysmex K1000; Sysmex, Germany). Differential leucocyte and reticulocyte counts were performed by microscopy. Serum iron concentration was determined using a colorimetric method (Randox Laboratories, Northern Ireland, UK), whereas serum ferritin and transferrin were measured by immunoturbidimetry (Randox). An enzyme-linked immunosorbent assay (ELISA) was used to measure soluble transferrin receptor (R&D Systems, Minneapolis, USA). Plasma levels of hepcidin-25 were quantified using a peptide enzyme immunoassay (Peninsula Laboratories, California). Serum C-reactive protein (CRP) was determined by nephelometry (Roche Diagnostics), serum interleukin (IL)-6 was evaluated by enzyme immunoassays (eBioscience, Austria). Serum albumin levels were measured using a colorimetric assay end-point method (Roche, Germany). Haptoglobin phenotype was determined by native polyacrylamide gel electrophoresis (PAGE) using N,N,N′,N′-tetramethylphenylenediamine (TMPD) staining, as previously described.[9]

Haptoglobin phenotype distribution among the 253 HD patients was 47 (18.6%) for Hp1-1, 135 (53.4%) for Hp2-1 and 71 (28.1%) for Hp2-2. No differences were found in Hp phenotype distribution between responder and non-responder HD patients ($P>0.05$). Moreover, no significant difference was found in all analysed variables when the results obtained were stratified only by Hp phenotype (data not shown). When the results were stratified by Hp phenotype and by response to rhEPO therapy, no significant differences were found for all analysed variables (Table I) except for serum iron. Indeed, non-responder HD patients with Hp 2-2 phenotype showed significantly lower iron serum levels ($P=0.007$) and a trend towards lower transferrin saturation ($P=0.085$) when compared with those who presented with the Hp1-1 phenotype (Table I). No difference was found when the patients were stratified by Hp phenotype and gender.

As previously mentioned, HD non-responders to rhEPO therapy present with a 'functional' iron deficiency. This is characterised by the presence of adequate iron stores, as defined by conventional criteria, but with an apparent inability to mobilise iron to adequately support erythropoiesis. The HD patients present higher serum

Table I. Clinical and haematological data, iron metabolism, inflammatory and nutritional markers, according to response to rhEPO therapy and Hp genotype.

	Responder HD patients (n=201)			
	Genotype 1-1 (n=39)	Genotype 1-2 (n=106)	Genotype 2-2 (n=56)	
Dialysis adequacy				
Dialysis period (years)	4.87±6.42	4.71±5.62	3.14±3.67	
Kt/Ve	1.43±0.21	1.44±0.25	1.52±0.27	
URR (%)	75.22±4.63	75.21±6.91	75.80±5.83	
Darbopoietin-α (micro/Kg)	0.52 (0.33–0.77)	0.41 (0.19–0.68)	0.37 (0.23–0.64)	
Haematological data				
Haemoglobin (g/dL)	11.72±1.13	11.89±1.56	11.75±1.28	
Haematocrit (%)	36.04±3.69	36.58±4.81	35.94±3.87	
Erythrocytes (x10^{12}/L)	3.75±0.44	3.82±0.55	3.77±0.49	
MCV (fL)	96.40 (94.40–99.53)	95.90 (93.20–98.80)	96.50 (93.32–99.70)	
MCH (pg)	31.40 (30.10–32.65)	31.50 (29.93–32.68)	31.60 (29.88–32.60)	
MCHC (g/dL)	32.54±1.05	32.57±1.21	32.73±1.41	
RDW (%)	13.60 (13.40–14.80)	14.20 (13.70–15.03)	14.85 (14.00–15.55)	
Reticulocytes (x10^9/L)	58.17±33.12	53.82±32.56	50.06±29.13	
RPI	1.08±0.60	1.01±0.65	0.96±0.59	
Platelets (x10^9/L)	155.34±56.86	186.08±67.42	180.86±54.39	
Leuccytes (x10^9/L)	5.93±1.52	6.51±1.92	6.33±1.75	
Neutrophils (x10^9/L)	3.60±1.04	4.02±1.45	3.91±1.51	
Lymphocytes (x10^9/L)	1.68±0.59	1.73±0.70	1.65±0.58	
Iron metabolism				
Iron (µg/dL)	52.21±25.32	50.73±26.19	59.45±34.89	
Transferrin (mg/dL)	176.00 (157.00–200.00)	179.50 (160.00–194.75)	182.00 (164.50–205.50)	
Transferrn saturation (%)	20.39±9.99	20.53±11.69	23.03±14.94	
sTfR (nmol/L)	21.19±7.18	22.25±9.88	20.98±11.85	
Ferritin (ng/mL)	443.70 (334.00–511.00)	443.95 (310.78–544.70)	456.30 (329.20–571.05)	
Hepcidin-25 (ng/mL)	1132.23 (736.94–1806.29)	1461.46 (695.92–2571.33)	1688.95 (1074.25–2070.11)	
Inflammatory markers				
CRP (mg/dL)	3.45 (2.08–15.74)	5.76 (2.13–11.64)	3.96 (1.97–7.75)	
IL-6 (pg/mL)	2.38 (1.37–4.34)	2.55 (1.43–4.79)	2.67 (1.51–5.80)	
Nutritional markers				
Albumin (g/dL)	3.95±0.41	3.99±0.32	4.00±0.30	
BMI (Kg/m^2)	26.16±5.02	26.12±4.64	25.80±4.24	

Differences between groups were analysed using Student's *t*-test or Mann-Whitney test, depending on the results obtained in the Kolmogorov-Smirnov test. Normally distributed variables are presented as mean±SD and those non-normally distributed are presented as median (interquartile range).

hepcidin levels, as well as higher chronic inflammation and reduced iron mobilisation.[8] This increased hepcidin expression during inflammation explains sequestration of iron in the macrophages and inhibition of intestinal iron absorption, the two hallmarks of anaemia of inflammation.

In the present study, the authors demonstrate that the Hp 2-2 phenotype is associated with a decrease in serum iron levels in non-responder HD patients, independently of the inflammatory process, as no differences were found in inflammatory markers, Hp phenotype or serum hepcidin levels between the three Hp phenotypes.

Hp 2-2 phenotype has been associated with increased iron retention in monocytes/macrophages, reducing the iron mobilisation needed to support erythropoiesis. The HD non-responders to rhEPO therapy who presented with the Hp 2-2 phenotype showed a decreased in serum iron levels, which could suggest the need for higher doses of rhEPO to achieve the target haemoglobin level.

In conclusion, the data presented show that HD non-responder patients with an Hp2-2 phenotype had decreased serum iron levels that appear to induce a weak response to rhEPO therapy.

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	Non-responder HD patients (n=52)			
P	Genotype 1-1 (n=8)	Genotype 1-2 (n=29)	Genotype 2-2 (n=15)	*P*
0.196	2.75±2.38	4.83±6.22	4.02±3.64	0.667
0.193	1.43±0.07	1.36±0.28	1.52±0.36	0.443
0.869	75.60±2.12	71.78±7.79	75.64±8.89	0.487
0.352	2.33 (2.20-2.43)	2.11 (1.72–2.71)	2.55 (1.87–3.72)	0.168
0742	9.64±1.65	10.88±1.66	10.95±1.58	0.138
0.622	30.58±3.90	34.16±4.92	33.84±5.28	0.187
0.661	3.31±0.57	3.66±0.58	3.78±0.65	0.210
0.814	91.42 (88.13–96.95)	96.00 (88.93–99.48)	91.96 (87.33–96.68)	0.857
0.832	28.61 (26.38–31.18)	31.00 (28.55–32.58)	29.10 (28.03–30.60)	0.667
0.684	31.20±2.73	31.73±1.72	30.94±1.89	0.407
0.067	17.50 (15.50–18.70)	17.00 (15.50–18.50)	17.00 (16.00–18.00)	0.663
0.486	65.61±39.59	62.15±30.13	71.53±27.12	0.619
0.712	0.84±0.60	0.84±0.44	1.01±0.39	0.545
0.076	200.13±78.57	188.52±70.78	192.79±99.28	0.937
0.232	5.59±1.75	6.33±2.57	7.38±2.70	0.210
0.276	3.80±1.41	4.19±2.08	5.09±2.20	0.247
0.707	1.25±0.68	1.45±0.61	1.61±1.04	0.543
0.179	74.13±45.07	54.10±29.21	33.73±11.06*	0.007
0.468	164.50 (148.25–221.75)	164.50 (140.75–194.00)	164.50 (138.00–212.50)	0.960
0.441	27.89±15.55	23.06±13.05	16.14±8.61	0.085
0.794	30.47±10.60	31.57±13.27	34.66±15.31	0.787
0.580	401.55 (281.33–629.75)	357.40 (236.98–558.10)	409.55 (242.53–592.43)	0.683
0.237	990.00 (110.00–1691.20)	1330.00 (570.00–2160.00)	744.65 (408.74–1368.26)	0.377
0.657	12.30 (2.69–21.93)	10.80 (4.62–39.00)	11.73 (4.36–41.39)	0.691
0.710	6.58 (4.30–19.55)	7.80 (3.69–14.84)	7.91 (5.16–14.23)	0.867
0.780	3.80±0.57	3.60±0.51	3.68±0.52	0.693
0.905	22.27±2.93	24.01±5.47	21.98±4.16	0.444

*<0.05 *vs.* genotype 1-1.

Kt/Ve: dialyser clearance of urea by dialysis time/volume of distribution of urea; URR: urea reduction ratio; MCV: mean cell volume; MCH: mean cell haemoglobin; MCHC: mean cell haemoglobin concentration; RDW: red cell distribution width; RPI: reticulocyte production index; sTfR: soluble transferrin receptor; CRP: C-reactive protein; IL-6: interleukin-6; BMI: body mass index.

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