Bilirubin levels are associated with beneficial effects in end-stage renal disease patients under hemodialysis

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Introduction

Cardiovascular disease (CVD) events are the most common cause of death in end-stage renal disease (ESRD) patients. Bilirubin has been proposed as a potential physiological antioxidant and anti-inflammatory agent that could have a protecting effect in oxidative stress conditions, such CVD, inflammatory diseases and cancer. The UGT1A1 gene locus is the most common genetic variant that affects the glucuronidation of bilirubin (TA duplication polymorphism in the TATA box region of the gene promoter). Homozygosis for the TA duplication is the main cause of Gilbert syndrome in Caucasian population and justifies some of the inter-individual variations in bilirubin levels, even in the normal population.

Our aim was, by screening for the duplication TA in the promoter region of UGT1A1 gene, to understand how this polymorphism, associated to increasing levels of bilirubin could have a protective effect in cardiovascular disease, in ESRD patients under haemodialysis (HD).

Material and methods

Subjects

This transversal study included 191 ESRD Portuguese patients under HD (105 males and 86 females, mean age: 66.13 years; standard derivation [SD]: 14.02 years). Patients with malignancy, autoimmune disease and with inflammatory or infectious diseases were excluded. Patients were under therapeutic HD three times per week for the duration of 3-5 hours, for a median time of 2.13 (0.82-5.24) years. For the HD procedure, high-flux polysulfone FX-class dialyzer of Fresenius (Bad Hamburg, Germany) was used.

Hematologic and biochemical assays

Laboratorial evaluation includes, hematological and dialysis adequacy, lipid profile, iron metabolism and inflammatory markers.

Screening for the duplication TA in the promoter region of UGT1A1 gene Genomic DNA was extracted from white blood cells (buffy coat) by proteinase K/salt precipitation method [34, 35]. Genotyping TA duplication in the TATA box of the UGT1A1 promoter was performed by polymerase chain reaction (PCR). Amplification reaction was carried out in a thermocycler (MiniOpticon™Real-Time PCR Detection System; Biorad) using 2µl of DNA, 0.5µl of each primer at a concentration of 10 pmol (forward: 5´-TAACTTGGTGTATCGATTGGTTTTTG-3´; reverse: 5´-ACAGCCATGGCGCCTTTGCT-3´) and 7.5 µl of PCR Master mix Promega (M750B) and water for a final volume of 15µl. The first step of PCR was 95°C denaturation for 5 minutes; followed by 35 cycles: denaturation at 95°C for 30 second, annealing at 55°C for 30 seconds, extension at 72°C for 45 seconds; final extension at 72°C for 10 minutes. PCR was followed by electrophoresis in 15% polyacrylamide gel in a Tris/Borate/EDTA buffer; the gel was stained with silver nitrate and photographed.

Results

The UGT1A1 genotype frequencies in HD patients were 49.2%, 42.4% and 8.4% for 6/6, 6/7 and 7/7 genotypes, respectively. Comparing the results for HD patients in the 1st tertile of bilirubin with those in the 3rd tertile, we found that the latter showed a significant decrease in platelet, leukocyte and lymphocyte counts; an increase in serum iron and in transferrin saturation, and a decrease in transferrin; a significant decrease in ox-LDL, ox-LDL/LDLc ratio, Lp (a), Apo A, Apo B and a significant increase in Apo A/Apo B ratio; a significant increase in adiponectin and paraoxonase 1 and a significant decrease in IL-6 serum levels, were observed. We also found statistically significant correlations between total serum bilirubin and adiponectin (r=0,238; p=0,001), transferrin (r= -0,213; p=0,003), iron (r=0,201; p=0,005), transferrin saturation (r=0,307; p<0,001), Apo A (r= -0,249; p<0,001), lymphocyte count (r= -0,223; p=0,002) and IL-6 (r= -0,193; p=0,008).

		Tertiles of total bilirubin levels			
	All patients (n=191)	T1 TB: <0.21 mg/dL (n=67)	T2 TB: 0.21 to 0.3 mg/dL (n=61)	T3 TB: >0.3 mg/dl (n=63)	P value[c]
Sociodemographic data and dialysis adequacy					
Age (years)	66.1 ± 14.0	67.7 ± 13.6	65.5 ± 15.5	65.1 ± 13.0	0.511
Gender (male), n (%)	105 (55)	33 (49.3)	31 (50.8)	41 (65.1)	0.142
Kt/ V urea	1.5 ± 0.3	1.5 ± 0.4	1.5 ± 0.2	1.4 ± 0.2	0.360
Creatinine (mg/dL)	8.1 ± 2.8	7.7 ± 2.4	8.2 ± 2.6	8.5 ± 3.3	0.306
Urea Reduction Ratio (%)	76.0 ± 6.6	76.2 ± 7.6	76.8 ± 6.2	75.1 ± 5.6	0.377
Dose of darbopoetin-α (µg/kg/week)	0.4 (0.2 - 0.7)	0.5 (0.3 - 0.8)	0.4 (0.2 - 0.6)	0.5 (0.2 - 0.9)	0.311
BMI (kg/m^2)	25.9 ± 4.6	26.3 ± 5.4	26.1 ± 4.1	25.3 ± 4.2	0.370
UGT1A1 genotype					
6/6, n (%)	94 (49.2)	33 (35.1)	31 (38.3)	3 (18.8)	
6/7, n (%)	81 (42.4)	35 (37.2)	20 (24.7)	6 (37.5)	0.236
7/7, n (%)	16 (8.4)	26 (27.7)	30 (37.0)	7 (43.8)	
Hematological data					
Hemoglobin (g/dL)	11.7 ± 1.4	11.4 ± 1.5	12.0 ± 1.4 a)	11.8 ± 1.3	0.042
Hematocrit (%)	36.4 ± 4.6	35.3 ± 4.5	37.5 ± 4.7 a)	36.6 ± 4.3	0.026
Erythrocytes (x10^{12} /L)	3.8 ± 0.5	3.7 ± 0.5	4.0 ± 0.5 a)	3.8 ± 0.5	0.043
Reticulocytes (x10^9/L)	49.3 (27.4 - 72.8)	56.0 (28.5 - 75.6)	40.1 (28.3 - 66.8)	50.8 (26.2 - 74.7)	0.318
RPI	0.9 (0.5 - 1.4)	0.9 (0.5 - 1.3)	0.8 (0.5 - 1.4)	1.1 (0.5 - 1.5)	0.834
Platelets (x10^9/L)	183.7 ± 55.1	197.7 ± 59.9	175.8 ± 43.6	164.0 ± 53.6 a)	0.027
White blood cells (x10^9/L)	6.4 ± 2.0	6.9 ± 2.0	6.3 ± 2.0	6.0 ± 1.9 a)	0.046
Neutrophils (x10^9/L)	4.0 ± 1.5	4.2 ± 1.3	3.8 ± 1.6	3.8 ± 1.6	0.247
Lymphocytes (x10^9/L)	1.7 ± 0.7	1.8 ± 0.9	1.7 ± 0.6	1.5 ± 0.5 a)	0.011
Neutrophil/Lymphocyte ratio	2.3 (1.8 - 3.3)	2.3 (1.8 - 3.4)	2.2 (1.5 - 3.0)	2.5 (2.0 - 3.3)	0.119
Iron metabolism					
Iron (mg/dL)	38.0 (30.0 - 54.0)	36.0 (29.0 - 43.0)	41.0 (29.0 - 55.0)	44.0 (32.0 - 56.0) a)	0.005
Transferrin (mg/dL)	184.3 ± 35.6	193.3 ± 34.6	182.4 ± 39.4	176.4 ± 31.1 a)	0.023
Transferin saturation (%)	17.7 ± 10.8	13.9 ± 6.2	17.7 ± 9.4	21.5 ± 14.1 a)	<0.001
sTfR (nmol/L)	23.3 ± 11.9	21.7 ± 9.4	25.0 ± 14.2	23.4 ± 11.7	0.281
Ferritin (ng/mL)	402.0 ± 152.6	369.3 ± 153.9	419.4 ± 162.7	419.8 ± 137.0	0.093
Hepcidin-25 (ng/mL)	1599.1 (863.6 – 2409.0	1738.0 (1144.3-2637.7)	1649.8 (931.7-2440.1)	1476.6 (537.8-2350.0)	0.108
Lipid profile					
Total cholesterol (mg/dL)	154.4 ± 43.4	156.7 ± 34.6	151.3 ± 35.1	155.0 ± 57.3	0.777
Trigliceride (mg/dL)	119.0 (91.0 - 176.0)	135.0 (91.0 - 183.0)	113.0 (96.5 - 172.5)	109.0 (85.0 - 151.0)	0.156
HDL-cholesterol (mg/dL)	42.3 ± 13.5	41.7 ± 12.1	42.5 ± 13.6	42.7 ± 14.9	0.908
LDL-cholesterol (mg/dL)	73.5 ± 29.5	73.8 ± 28.5	73.2 ± 28.0	73.4 ± 32.2	0.992
ox-LDL (U/L)	36.0 ± 15.4	39.8 ± 21.3	34.5 ± 8.6	33.2 ± 12.0 a)	0.033
ox-LDL/LDLc ratio (U/mg)	0.053 ± 0.019	0.058 ± 0.025	0.051 ± 0.013 a)	0.048 ± 0.013 a)	0.007
Lp (a) (mg/dL)	45.4 (25.2 - 88.1)	50.4 (27.3 - 106.2)	45.4 (24.1 - 89.1)	37.4 (24.7 - 68.2)a)	0.052
Apo A (mg/dL)	123.1 ± 30.5	128.9 ± 32.4	125.7 ± 32.7	114.3 ± 23.8 a)	0.017
Apo B (mg/dL)	72.8 ± 21.8	81.8 ± 21.3	70.6 ± 18.9 a)	65.4 ± 31.9 a)	<0.001
Apo A/Apo B ratio	1.83 ± 0.67	1.68 ± 0.54	1.89 ± 0.74	1.85 ± 0.65 a)	0.048
Inflammatory markers					
Adiponectin (mg/L)	9.2 ± 4.7	7.9 ± 3.7 [b]	9.1 ± 4.6	10.7 ± 5.4 a)	0.003
PON1 (nmol p-nitrofenol/mL/min)	398.3 ± 92.6	376.0 ± 70.1	398.5 ± 92.9	418.6 ± 106.2 a)	0.040
CRP (mg/dL)	5.2 (2.3 – 13.3)	7.1 (3.2 - 14.6)	4.9 (2.1 - 13.5)	3.6 (1.9 - 12.7)	0.393
IL-6 (pg/mL)	2.3 (1.4 - 4.3)	3.1 (2.1 - 5.4)	2.1 (1.4 - 4.0) a)	1.6 (1.1 - 3.4) a)	0.001
Albumin (g/dL)	3.9 ± 0.4	3.9 ± 0.3	3.9 ± 0.4	3.9 ± 0.4	0.936

Conclusions

Our data suggest that higher bilirubin levels are associated with beneficial effects in ESRD patients under HD, by improving lipid profile and reducing the inflammatory grade, which might contribute to increase iron availability and protect for CVD events. Moreover, we describe for the first time, an association between high bilirubin levels, within the normal range, with higher adiponectin and paraoxonase 1 activities.

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