STWEAK AND PTX3 AS MARKERS OF ENDOTHELIAL (DYS)FUNCTION AND INFLAMMATION IN END-STAGE RENAL DISEASE PATIENTS UNDER HEMODIALYSIS

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INTRODUCTION

Despite the technical advances in the dialysis procedures, and the medical support to end-stage renal disease (ESRD) patients under hemodialysis (HD), a high morbidity and mortality due to cardiovascular events is still observed (about 50%). ESRD appears to represent a clinical model of accelerated atherosclerosis associated with chronic inflammation. The soluble form of TNF-like weak inducer of apoptosis (sTWEAK) and pentraxin 3 (PTX3), have, recently, been proposed as potential markers of atherosclerosis and inflammation. The aim of this study was to clarify the role of sTWEAK and PTX3 as markers of endothelial (dys)function and inflammation in ESRD patients under HD. We also intended to evaluate the potential of these markers in the response to rhEPO therapy, as non-responders patients, usually, present an enhanced inflammatory response.

MATERIALS AND METHODS

Patients: We evaluated 70 ESRD patients under HD and rhEPO [0.42 (0.20 - 0.75) microg/Kg/week] therapies and 21 healthy individuals. The classification of CKD patients as responders or non-responders to rhEPO therapy was performed in accordance with the European Best Practice Guidelines. The intravenous iron supplementation used, was based on the European Best Practice Guidelines for the management of anemia in patients under HD and rhEPO therapies. All participants gave their informed consent to participate in this study that was previously approved by the Ethics Committee of the Clinic of HD. Patients with autoimmune disease, malignancy, and acute or chronic infection were excluded. ESRD patients were under therapeutic HD 3/week, for 3–5 h, for a median of 2.11 (0.85 - 5.19) years. All patients used the high-flux polysulfone FX-class dialyzers of Fresenius. Dialysis clearance of urea was expressed as KT/V.

Assays: Blood samples were obtained immediately before HD procedure and processed within 2h of collection. Blood was collected to tubes with and without anticoagulant EDTA, in order to obtain plasma and serum. Aliquots were immediately stored at -80ºC until assayed.

Red blood cell (RBC) count, hematocrit, hemoglobin concentration, hematological indices [mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC)] and red cell distribution width (RDW) were measured by using an automatic blood cell counter (Sysmex K1000; Sysmex, Hamburg, Germany).

Concentration of interleukin (IL)-6, soluble transferrin receptor (sTfR), hepcidin -25, d-dimers and tPA were evaluated by using standard commercial ELISA kits (adiponectin and IL-6 – from Bender MedSystems; sTfR - R&D Systems; hepcidin -25 – Bachem, UK). Ferritin, transferrin, iron and C-reactive protein (CRP) were measured using latex-enhanced immunoturbidimetry (Ferritin and transferrin, Randox; iron and CRP, Tina-quant, Roche). Transferrin saturation (TS) was calculated: TS (%) = 70.9 x serum iron concentration in (µg/dL) / serum transferrin concentration in (mg/dL). The circulating levels of PTX3 and sTWEAK were evaluated by using standard commercial enzyme-linked immunoassays (Quantikine ELISA, Human Pentraxin 3, R&D Systems; Human TWEAK Instant ELISA, eBioscience).

STATISTICS

For statistical analysis, we used the Statistical Package for Social Sciences (SPSS), version 19.0. The distribution of continuous variables was analyzed by using Kolmogorov-Smirnov test, in order to assess significant departures from Normality. Normally distributed variables are presented as mean SD and those non-normally distributed are presented as median (interquartile range). Multiple comparisons by one-way ANOVA supplemented with Tukey's HSD post-hoc test or Chi-Square test. Comparisons between groups were performed using Students t-test whenever the parameters presented a Gaussian distribution and the Mann-Whitney U-test in the case of a non-Gaussian distribution. Significance was accepted at p less than 0.05.

RESULTS

Table I – Demographic, and dialysis-related data for the studied groups

	Controls (n=21)	Patients (n=70)	P*
Age (y)	37.40 ± 9.99	66.93 ± 14.89	<0.001
BMI (kg/m^2)	25.48 ± 4.79	22.07 ± 2.05	<0.001
Blood Pressure (mmHg):			
Diastolic	-	65.47 ± 9.80	-
Systolic	-	131.59 ± 19.43	-
Gender (Male/Female, %)	42.9 / 57.1	57.1 / 42.9	-
Hypertension (%)	-	55.7	-
Diabetics (%)	-	35.7	-
Responders/Non responders (%)	-	85.7 / 14.3	-
URR (%)	-	75.73 ± 7.0	-
KT/Ve	-	1.50 (1.30 – 1.60)	-
Ultrafiltration Volume (L)	-	2.46 ± 1.10	-
Time on dialysis (y)	-	2.15 (0.71 – 5.24)	-
Albumin (g/dL)	-	3.89 ± 0.38	-
Creatinin (mg/dL)	-	8.09 ± 3.25	-
Darbopoietin-α (µg/kg/week)	-	0.46 (0.18 – 0.91)	-

Table II – Hematological data, iron metabolism, inflammatory and endothelial markers

	Controls (n = 21)	Patients (n = 70)	*P**
Hematological data:			
Erythrocytes (x10 ^{12}/L)	4.92 ± 0.45	3.81 ± 0.61	<0.001
Hemoglobin (g/dL)	14.94 ± 0.85	11.53 ± 1.74	<0.001
Hematocrit (%)	44.37 ± 2.72	36.34 ± 5.43	<0.001
MCV (fL)	87.57 ± 12.71	95.59 ± 6.89	<0.001
MCH (pg)	30.47 ± 1.68	30.50 ± 2.33	0.96
MCHC (g/dL)	33.7 ± 0.46	32.00 ± 1.18	<0.001
RDW (%)	14.97 ± 0.54	15.16 ± 2.02	0.51
Iron Metabolism:			
Transferrin (mg/dL)	305 (278.5 – 329)	182.5 (162.5 – 209.5)	<0.001
Iron (mg/dL)	52 (36.5 – 72)	37.5 (28.0 – 55.0)	0.01
sTfR (nmol/L)	14.07 ± 3.89	23.65 ± 13.41	<0.001
Ferritin (ng/mL)	100.83 ± 61.1	416.75 ± 150.41	<0.001
Transferrin saturation (%)	12.1 (8.73 – 15.39)	14.50 (11.25 – 20.45)	0.03
Hepcidin (ng/mL)	223.34 (132.33 – 318.73)	1278.18 (718.07 – 1893.86)	<0.001
Inflammatory markers:			
IL-6 (pg/L)	0.41 (0.27 – 0.59)	2.5 (1.38– 5.00)	<0.001
CRP (mg/dL)	0.61 (0.42 – 2.49)	5.7 (2.6 – 15.13)	<0.001
PTX3 (ng/dL)	1.58 (0.83 – 2.32)	1.53 (0.93 – 2.17)	0.79
Endothelial markers:			
sTWEAK (pg/mL)	587.49 (506.36 – 661.61)	389.47 (337.42 – 457.03)	<0.001
tPA (ng/mL)	3.86 (2.63 – 5.55)	5.67 (3.85 – 8.54)	0.01
PAI-1 (ng/mL)	28.79 (15.11 – 43.86)	20.26 (12.37 – 31.58)	0.14
D-dímer (ng/mL)	0.26 (0.18 – 0.39)	0.67 (0.32 – 1.57)	<0.001

HD patients, compared with the control group, presented:

- A normochromic normocytic anemia
- Significant changes in iron metabolism:
  - lower iron and transferrin;
  - higher transferrin saturation, sTfR, ferritin and hepcidin serum levels;
- Significant changes in inflammatory markers:
  - higher CRP and IL-6;
  - no differences were found in PTX3 levels
- Significant changes in endothelial markers:
  - higher levels of D-dimers and tPA
  - higher levels of sTWEAK

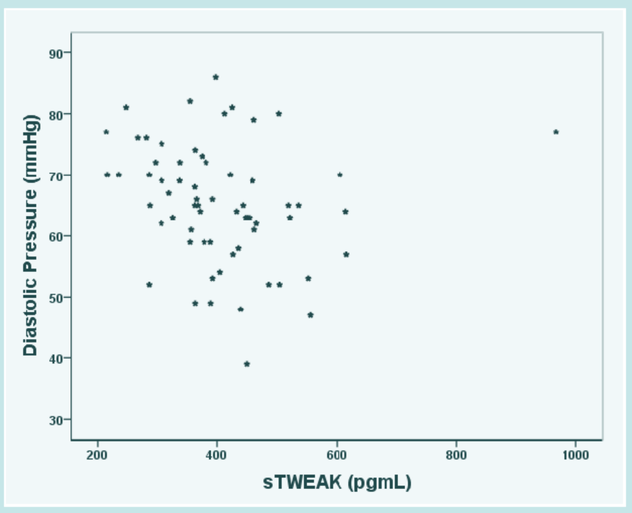

Figure 1 – Significant correlation between sTWEAK and diastolic pressure

In HD patients group:

- Significant correlations were found between PTX3 levels and:
  - IL-6 (r=0.408; p=0.002)
  - CRP (r=0.452; p<0.001)
  - iron (r=-0.403; p=0.002)
  - transferrin saturation (r=-0.280; p=0.037)
  - MCHC (r=-0.358; p=0.007)
  - RDW (r=0.416; p=0.006)
  - urea reduction ratio (r=0.301; p=0.028)
  - albumin (r=-0.449; p=0.001)
  - rhEPO doses (r=0.479; p<0.001)

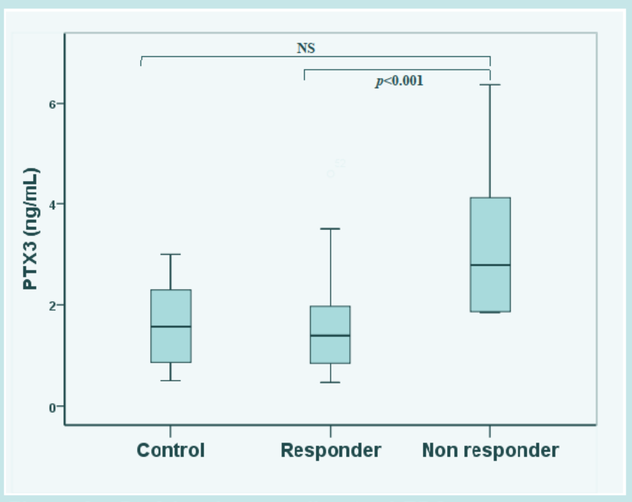

Figure 2 – Ptx3 levels according to rhEPO response

- Significant correlation were found between sTWEAK and diastolic blood pressure (r=-0.327; p=0.008) (Figure 1)
- Analyzing the results according to the response to rhEPO therapy, we found that inflammation was enhanced in resistant patients, as showed by the increase in PTX3 (Figure 2), CRP and IL-6 levels.

CONCLUSIONS

Our results showed that PTX3 is a good marker of resistance to rhEPO in ESRD patients under HD. Moreover, our results also showed that sTWEAK is a marker of endothelial dysfunction in our patients as showed by the positive correlation between sTWEAK and diastolic blood pressure.

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