Hemodialysis to online-hemodiafiltration change is associated to a decrease in circulating cell-free DNA levels

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INTRODUCTION/AIMS

Patients with end-stage renal disease (ESRD) undergoing hemodialysis (HD) are characterized by the presence of chronic inflammation, which contributes to the high morbidity and mortality of these patients. Recently, it was demonstrated that ESRD patients present increased levels of cell-free circulating DNA, probably due to the release of DNA from cellular necrosis and apoptosis. As cell –free DNA could lead to inflammation, and online-hemodiafiltration (OL-HDF) technique was proposed to associate a reduction in inflammation, we aimed to evaluate the effect of HD to OL-HDF change in cell-free circulating DNA serum levels.

METHODS

In this longitudinal study, 59 ESRD patients (31 males, 28 females; mean age 66.75±12.61 years), undergoing HD for median of 1.98 (1.08 – 3.88), were changed from HD to OL-HDF. Patients were evaluated before, and ten months after they changed to OL-HDF. Patients with evidence of autoimmune diseases, malignancy, and acute or chronic infectious were excluded. All participants gave their informed consent to participate in this study.

The levels of circulating free-cell DNA were evaluated directly in serum samples, as recently described by Goldstein et al. (Ann Clin Biochem 2009; 46:488-94). Briefly SYBR® Gold Nucleic Acid Gel Stain (Invitrogen, Paisley UK) was diluted in dimethyl sulfoxide and phosphate buffer; 10µL of the serum sample were mixed 40µL of SYBR® Gold solution. Fluorescence was measured with a 96-well fluorometer, at emission wavelenght of 535nm and excitation wavelenght of 485nm. Erythrocyte count, hematocrit, hemoglobin concentration and hematimetric indices [mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC)], total leukocyte and platelet counts, were measured by using an automatic blood cell counter (Sysmex K1000; Sysmex, Hamburg, Germany). Differential leukocyte counts were evaluated in Wright-stained blood films. Reticulocyte count was made by microscopic counting on blood smears after vital staining with New methylene blue (reticulocyte stain; Sigma, St. Louis, Mo., USA). The reticulocyte production index (RPI) was calculated as an appropriate way to measure the effective RBC production, by correcting for both changes in hematocrit(degree of anemia) and for premature reticulocyte release from the bone marrow. Serum iron concentration was determined using a colorimetric method (Iron, Randox Laboratories Ltd., North Ireland, UK), whereas serum ferritin and serum transferrin concentrations were measured by immunoturbidimetry (Ferritin, Laboratories Ltd., North Ireland, UK; Transferrin, Laboratories Ltd., North Ireland, UK). Transferrin saturation (TS) was calculated by the formula: TS (%) = 70.9 x serum iron concentration (µg/dL)/serum transferrin concentration (mg/dL). Enzyme-linked immunosorvent assays were used to measure serum soluble transferrin receptor (sTfR; human sTfR immunoassay, R&D Systems, Minneapolis, Minn., USA). Plasma levels of hepcidin-25 were quantified using a Peptide Enzyme Immunoassay (Bachem Group, Peninsula Laboratories, LLC, San Carlos, California). Serum C-reactive protein (CRP) was determined by nephelometry [CRP (latex) High-Sensitivity, Roche Diagnostics], plasma levels of elastase was evaluated by enzyme immunoassays (Human PMN Elastase ELISA, eBioscience, Vienna, Austria). Moreover, socio-demographic factors, haematological and dialysis adequacy data, iron metabolism and inflammatory markers were also evaluated.

RESULTS

HD to OL-HDF change was associated to an improvement in dialysis adequacy, as showed by the significant increase in Kt/Ve and URR. Concerning to hematological data, no differences were found in hemoglobin levels; however, a significant decrease in MCV, RPI, reticulocyte and platelet counts, and a significant increase in MCHC were found. Significant changes in iron metabolism were also observed, namely, an increase in iron, sTfR, and transferrin saturation, and a decrease in transferrin levels. After change from HD to OL-HDF our patients presented a significant decrease in circulating cell-free DNA levels [mean (SD), 8459.6 (1502.6)mg/mL; mean (SD), 7983.4 (1863.3) mg/mL, *P*=0.006] (Table 1). In OL-HDF patients, significant correlations were found between cell-free DNA serum levels and CRP (r=0.361, p=0.002), iron (r=-0.243, p=0.04), sTfR (r=0.260; p=0.027) and rhEPO doses (r=0.280; p=0.017).

Table 1. Hematological and biochemical data for ESRD patients under HD, and after changing to OL-HDF.

Parameters	HD (n=59)	OL-HDF (n=59)	*P**
Hematological parameters			
Erythrocytes, $x10^{12}$/L	3.68±0.66	3.74±0.50	0.426
Hemoglobin, g/dl	11.57±1.30	11.55 ±1.46	0.925
Hematocrit, %	34.97±5.61	34.84±4.29	0.851
MCV, fl	94.28±12.53	93.51±5.79	<0.001
MCH, pg	30.83±4.37	30.97±2.26	0.362
MCHC, g/dl	32.23±3.92	33.15±0.96	<0.001
RDW, %	14.30 (13.50 - 15.30)	14.20 (13.60 - 14.90)	0.496
Platelets, $x10^9$/L	183.23 ± 50.80	172.27 ± 53.48	0.020
Reticulocytes, $x10^9$/L	38.40 (15.92 - 62.08)	32.40 (17.08 - 48.75)	0.189
RPI	0.75 (0.40-1.23)	0.50 (0.30-0.80)	<0.001
Lekocytes, $x10^9$/L	6.25±1.76	6.20±1.77	0.812
Lymphocytes, $x10^9$/L	2.55±1.14	1.67±1.66	0.181
Neutrophils, $x10^9$/L	2.55±1.14	3.80±1.30	0.229
Lymphocyte:Neutrophil ratio	2.41±1.27	2.55±1.14	0.151
Iron metabolism markers			
Iron, mg/dl	43.00 (34.00 - 56.00)	69.00 (56.00 - 89.00)	<0.001
Ferritin, ng/ml	458.10 (291.50 - 541.00)	463.00 (305.40 - 567.50)	0.450
sTfR, nmol/l	17.85 (13.20 - 24.13)	22.02 (16.60 - 25.85)	0.005
Transferrin, mg/dl	192.00 (176.00 - 212.00)	140.00 (126.00 - 163.00)	<0.001
Transferrin saturation, %	16.06 (12.08 - 20.94)	33.11 (25.57 - 45.99)	<0.001
Hepcidin -25, ng/ml	702.24±347.38	625.22±330.09	0.093
Inflammatory markers			
Cell free DNA	8459.6 ± 1502.6	7983.4 ± 1863.3	0.006
CRP, mg/ml	5.50 (2.76 - 10-54)	5.65 (3.11 – 56-41)	0.829
dialysis adequacy			
Duration of session	4hours/ 3 sessions/ week	4hours/ 3 sessions/ week	
rhEPO (U/Kg/week)	0.4 (0.2 – 0.6)	0.3 (0.2 – 0.5)	0.691
Kt/Ve	1.53±0.24	1.72±0.84	0.121
URR (%)	77.07±5.45	79.33±6.77	0.005

For statistical analysis was used Statistical Package for Social Science version 17.0. Parameters are expressed as mean ± SD or as median values (inter quartile range) when appropriate.

*To compare data from patients on HD and after change to ON-HDF was used Wilcoxon test. Significance was accepted at $P < 0.05$. MCV: mean cell volume; MCH: mean cell hemoglobin; MCHC: mean cell hemoglobin concentration; RDW: red cell distribution width; RPI: reticulocyte production index; sTfR: soluble transferrin receptors; CRP: C-reactive protein, rhEPO: recombinant human erythropoietin; URR: urea reduction ratio

CONCLUSIONS

According our data OL-HDF may contribute to improvement in erythropoiesis due to higher iron availability as a result of decrease in cell free DNA levels and inflammation markers generally. Moreover, OL-HDF patients present correlations between circulating cell-free DNA levels with inflammation and iron metabolic disturbances, as well as with rhEPO doses, suggesting that a decreased in cell-free DNA could be related to a decreased in cellular necrosis, apoptosis, and in inflammation. Switch to OL-HDF also resulted in better dialysis adequacy and seems to have beneficial effects for the treatment of ESRD patients comparing to conventional HD. OL-HDF seems to be well –tolerated treatment which could be applied regularly in ESRD patients.

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Flavio Reis
DOI: 10.3252/pso.eu.50era.2013
Poster presented at: ERA-EDTA 50th Congress Istanbul, Turkey May 15-21, 2013
PosterSessionOnline.com