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Short communication

Study of association between interleukin-17 and interferon-gamma and recombinant human erythropoietin dose in patients undergoing peritoneal dialysis

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ABSTRACT

Background: A common complication in patients undergoing peritoneal dialysis (PD) is a chronic inflammatory state and anemia that can be treating by recombinant human erythropoietin (rHuEPO). Higher required dose of rHuEPO could be expected in patients with higher cytokine levels. Additionally, it is known that peritoneal inflammation can be correlated with systemic inflammation and this could contribute to the compromised rHuEPO required dose in anemic patients with end stage renal disease (ESRD). Thus, the current study aimed to evaluate the association between levels of systemic and local interferon (IFN)-γ, interleukin (IL)-17 and other cytokines and the dose of rHuEPO used by patients undergoing PD for the correction of anemia.

Methods: Thirty-one patients under PD using rHuEPO were evaluated in this cross-sectional study. Plasma and dialysate levels of IL-2, IL-4, IL-6, IL-10, IL-17, tumour necrosis factor (TNF)-α and IFN-γ were determined using the Cytometric Bead Array TM kit (CBA; BD Bioscences, San Jose, CA). The relation between the levels of each cytokine levels and the tertiles of rHuEPO were plotted on box-plot graphics and then the medians of interleukins levels were compared by median comparison test. The significance level adopted was 5% and the analysis was performed by the softwares STATA (version 12.0) and GraphPad Prism 3.0.

Results: The median of IL-17 and IFN-γ plasma levels were significant higher in the group with higher rHuEPO dosage. However, this association was not observed in the dialysate levels, as well as was not observed a relationship between the other plasma and dialysate cytokines evaluated in this study and the dose of rHuEPO.

Conclusions: Our study found increased IL-17 and IFN-γ plasma, but no dialysate levels, in patients receiving higher doses of rHuEPO, suggesting may exist a relationship between systemic inflammation of ESRD, and the necessary levels of rHuEPO for the correction of anemia in these patients.

1. Introduction

Peritoneal dialysis (PD) is a renal replacement therapy that involves the flow of blood, the peritoneal membrane (PM) and the dialysis solution. The blood filtration is done using the PM via an intraperitoneal catheter. The solute molecules diffuse through a semipermeable membrane, the peritoneum, passing the side of higher concentration to lower concentration [1].

A common complication in patients undergoing PD, as well as in end stage renal disease (ESRD) is anemia. The anemia of chronic disease is a condition that have an important impact in ESRD and the main cause is the inadequate synthesis of erythropoietin (EPO) [2]. Anemia affects cognitive function, quality of life, exercise capacity, and cardiac function in patients with ESRD [3]. The introduction of recombinant human erythropoietin (rHuEPO) in clinical practice in 1986 completely changed the monitoring of patients

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with ESRD. A correction of anemia by rHUEPO contributes to greater patient survival, however, has become a permanent challenge in medical conduct, once some patients experience erythropoiesis-stimulating agents hyporesponsiveness [1,4].

The molecular mechanisms underlying this hyporesponsiveness remain unclear, however it has been postulated that immune activation and production of proinflammatory cytokines have play an important role [5,6]. In support of this hypothesis, the proinflammatory cytokines, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and interleukin (IL)-1 have been shown to suppress erythropoiesis *in vitro* [1]. Some studies support the hypothesis that IL-17 may play a role in anemia of chronic disease in which prolonged immune stimulation and increased IL-17 production may inhibit the burst forming unit-erythroid (BFU-E) [5,7]. In the same way, IFN-γ also has a strong impact on bone marrow during inflammation, as it affects the differentiation of most hematopoietic progenitor cells [6,8].

Based on this statement, the hypothesis of our study was that higher required dose of rHuEPO could be expected in patients with higher cytokine levels. Additionally, it is known that peritoneal inflammation is correlated with systemic inflammation and this could contribute to the compromised rHuEPO required dose in anemic patients with ESRD [9].

Thus, the current study aimed to evaluate the association between levels of systemic and local IFN-γ, IL-17 and other cytokines and the dose of rHuEPO used by patients undergoing PD for the correction of anemia.

2. Methods

2.1. Study design and population

This is a cross-sectional study performed with 31 patients under PD by at least 90 days. All patients were receiving rHuEPO to treat the anemia of kidney disease. Patients were treated at Nephrology Centre of the Sao João de Deus Hospital in Divinópolis, Minas Gerais, Brazil. Exclusion criteria were presence of acute diseases, autoimmune diseases, neoplasia, HIV positive, presence of an episode of peritonitis one month before or after the evaluation, presence of pregnancy, incapable to sign the consent form due to psychiatric condition or mental disorder.

2.2. Ethical considerations

The study was approved by the Ethics Committee of Research from Sao João de Deus Hospital and Federal University of Sao João del Rei. All patients signed the consent form to join the study.

2.3. Blood and dialysate

The venous blood samples (5 mL) were collected from all study participants using polyethylene syringes and transferred to tubes containing anti-coagulant ethylenediaminetetraacetic acid (EDTA). In order to separate the plasma, the samples were centrifuged 2000*g* for 15 min using a Novatecnica® centrifuge model NT815. Sample of dialysate (10 mL) was collected from the drained volume during the peritoneal equilibration test performance, using sterile flask and stored in a −80 °C freezer. Immediately before starting the measurements, the samples were thawed in a water bath at 37 °C.

2.4. Plasmatic cytokines assay

IL-2, IL-4, IL-6, IL-10, IL-17, IFN-γ and TNF-α were determined using a Cytometric Bead Array TM kit (CBA; BD Biosciences, San Jose, CA). Plasma and dialysate samples were mixed to capture specific beads for each cytokine. Anti-IL-2, anti-IL-4, anti-IL-6, anti-IL-10, anti-IFN-γ and anti-TNF-α antibodies were added, conjugated with phycoerythrin and incubated for 2 h under room temperature, protected from light. Tubes were then centrifuged (200*G* for 5 min) and the supernatant was carefully aspired and discarded. The pellets containing beads were resuspended and the samples were analysed on the BD LSR Fortessa (BD Company, San Diego, CA) cytometer. The data obtained were analysed by the BD ™ Cell Quest and FCAP Array softwares. Results were expressed in mean fluorescence intensity (MFI) for all cytokines analysed.

2.5. Study variables

The response variables were plasma e dialysate cytokine levels (continuous variables). The dose of rHuEPO/kg/week was categorized using tertiles: first (<95.52 rHuEPO/kg/week), second (>95.52 and <111.94 rHuEPO/kg/week) and third (>111.94 rHuEPO/kg/week).

2.6. Statistical analysis

Initially, the sociodemographic and clinical characteristics of participants were expressed as proportions for categorical variables and medians with interquartile ranges for continuous variables. The relation between the levels of cytokines and the

Table 1
Distribution of the study population according the sociodemographic and clinical characteristics.

Characteristics	Patients (n = 31)
Age (years old)	61.3 (16.6)
Gender	
Male [n(%)]	15 (48.4)
Female [n(%)]	16 (51.6)
BMI (kg/m^2)	24.8 (5.5)
ESRD primary causes [n(%)]	
Hypertensive nephrosclerosis	5 (16.1)
Diabetic nephropathy	12 (38.7)
CGN	8 (25.8)
Polycystic renal disease and kidney and urinary tract congenital alterations	5 (16.1)
Unknown etiologies	1 (3.3)
Blood pressure	
Systolic pressure (mmHg)	148.0 (18.8)
Diastolic pressure (mmHg)	80 (80–90)
Peritoneal dialysis time (months)	36 (12–48)
Medicine use	
β-Blockers	15 (48.4%)
Calcium channel antagonist	11 (35.5%)
Angiotensine receptor antagonist	17 (54.8%)
ACE inhibitor	2 (6.5%)
Diuretics	23 (74.2%)
Anxiolytics/antidepressives	9 (29.0%)
Vitamin supplementation	14 (45.2%)
Outros (acetylsalicylic Acid, statins, insulin)	21 (67.7%)
Red blood cell count × 10^6/mL	3.7 (3.4–3.8)
Haemoglobin (g/dL)	10.8 (1.1)
Haematocrit (%)	34.1 (3.6)
MCV	93.2 (5.2)
MCH	29.9 (1.7)
MCHC	32 (31.7–33.0)
Platelets count × 10^3/mL	223.0 (186.0–292.0)
White blood cell count × 10^3/mL	6.9 (2.4)
Serum iron (μg/dL)	63.1 (25.6)
IBTC	207 (188–238)
TSI (%)	30.7 (14.7)
Ferritin (ng/mL)	266 (125–451)

The distribution was made by percentage (%), mean and standard deviation or medians and interquartile ranges. BMI: Body Mass Index; ESRD: End Stage Renal Disease; CGN: Chronic Glomerulonephritis; ACE: Angiotensin-converter enzyme. MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Haemoglobin; MCHC: Mean Corpuscular Haemoglobin Concentration; IBTC: Iron Binding Total Capability; TSI: Transferrin saturation index.