UNIVERSIDADE FEDERAL DE ALFENAS – UNIFAL-MG

ROBSON EUGÊNIO DA SILVA

RELATIONSHIP BETWEEN CYTOKINES AND CHEMOKINES SYSTEMIC LEVELS, CREATININE CLEARANCE AND DIALYSIS DOSE ADEQUACY IN PATIENTS WITH END-STAGE RENAL DISEASE ON HEMODIALYSIS

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Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biociências Aplicadas à Saúde pela Universidade Federal de Alfenas (UNIFAL-MG. Área de concentração: Fisiopatologia. Orientador: Prof. Dr. Rômulo Dias Novaes.

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ABSTRACT

The accumulation of uremic toxins is associated to systemic inflammation and mortality in patients with end-stage renal disease (ESRD) undergoing hemodialysis (HD). Although the dialysis dose modulates the clearance of low molecular weight toxins, the relationship between dialysis adequacy and systemic inflammatory mediators is often overlooked. Thus, the relationship between dialysis adequacy and the levels of pro- and anti-inflammatory cytokines and chemokines were investigated. Forty-four volunteers (19 women and 25 men) with ESRD undergoing hemodialysis were investigated in this cross-sectional study. Age, body mass index, time in HD, nutritional status, Kt/V and blood biochemical parameters was similar in patients of both genders ($P>0.05$). Thus, patients were stratified by dialysis adequacy measured according Kt/V method (adequate $Kt/V \geq 1.2$). Post-HD urea, creatinine, cytokines (IFN-γ, IL-4 and IL-10) and chemokines (CCL-2, CCL-5, CXCL-8 and CXCL-10) were higher in patients with $Kt/V<1.2$ ($P<0.05$). Kt/V exhibited significant correlation with CXCL-10/IP-10 serum levels. Positive correlation between creatinine with IFN-γ, CCL-2/MCP-1, and CXCL-10/IP-10, and negative correlation with IL-10 was identified in patients with $Kt/V<1.2$ ($P<0.05$). In patients with $Kt/V \geq 1.2$, only IL-10 was positively and CXCL-10/IP-10 negatively correlated with creatinine levels ($P<0.05$). Kt/V and creatinine levels exhibited variable predictive value (Kt/V= 27% to 37%, creatinine= 29% to 47%) to explain cytokines and chemokines circulating levels in patients with adequate and inadequate dialysis dose. Taken together, our findings provide evidence that in addition to modulating uremic toxins levels, such as urea and creatinine, the dose of dialysis is associated with circulating levels of inflammatory mediators. Thus, low Kt/V results and creatinine accumulation are closed correlated with increased levels of proinflammatory cytokines and chemokines, as well as a reduction in anti-inflammatory cytokines.

Keywords: Hemodialysis; inflammation; kidney disease; pathology; uremic toxins.

RESUMO

O acúmulo de toxinas urêmicas está associado à inflamação sistêmica e mortalidade em pacientes com doença renal terminal (DRT) em hemodiálise (HD). Embora a dose de diálise module a eliminação de toxinas de baixo peso molecular, a relação entre a adequação da diálise e os mediadores inflamatórios sistêmicos costuma negligenciada. Assim, foi investigada a relação entre a adequação da diálise e os níveis de citocinas e quimiocinas pró e anti-inflamatórias. Quarenta e quatro voluntários (19 mulheres e 25 homens) com DRT em HD foram investigados neste estudo transversal. Idade, índice de massa corporal, tempo em HD, estado nutricional, Kt/V e parâmetros bioquímicos sanguíneos foram semelhantes em pacientes de ambos os sexos (P>0,05). Assim, os pacientes foram estratificados pela adequação da diálise de acordo com o Kt/V (Kt/V adequado ≥1,2). Ureia pós-HD, creatinina, citocinas (IFN-γ, IL-4 e IL-10) e quimiocinas (CCL-2, CCL-5, CXCL-8 e CXCL-10) foram mais elevados em pacientes com Kt/V <1,2 (P <0,05). Kt/V exibiu correlação significativa com os níveis séricos de CXCL-10 / IP-10. Correlação positiva entre creatinina com IFN-γ, CCL-2 / MCP-1 e CXCL-10 / IP-10, e correlação negativa com IL-10 foi identificada em pacientes com Kt/V <1,2 (P <0,05). Em pacientes com Kt/V ≥1,2, apenas IL-10 foi positivamente e CXCL-10/IP-10 negativamente correlacionados com os níveis de creatinina (P<0,05). Os níveis de Kt/V e creatinina exibiram valor preditivo variável (Kt/V= 27% à 37%, creatinina= 29% à 47%) para explicar os níveis circulantes de citocinas e quimiocinas em pacientes com dose adequada e inadequada de diálise. Em conjunto, nossos achados indicaram que além de modular os níveis de toxinas urêmicas, a dose de diálise está associada aos níveis circulantes de mediadores inflamatórios. Assim, baixo valores de Kt/V e acúmulo de creatinina são correlacionados e preditores úteis de níveis aumentados de citocinas e quimiocinas pró-inflamatórias, bem como de reduzidos níveis de citocinas anti-inflamatórias em pacientes em HD.

Palavras-chave: Doença renal; hemodiálise; inflamação; patologia; toxinas urêmicas.

SUMÁRIO

1 REVISÃO DE LITERATURA

1.1 Doença renal crônica: classificação e epidemiologia

A doença renal crônica (DRC) constitui um termo genérico utilizado para caracterizar desordens que afetam a estrutura e função dos rins (KALANTAR-ZADEH *et al*, 2021; LEVEY & CORESH, 2012). A DRC é definida como a presença de lesão renal que cursa com albuminúria persistente (>30 mg/g) ou redução da função renal com taxa de filtração glomerular (TFG) inferior a 60 ml/min/1,73m^2 da área de superfície corporal durante 3 meses ou mais (LEVEY & CORESH, 2012). A DRC apresenta desenvolvimento progressivo e os estágios iniciais da doença são muitas vezes assintomáticos, sendo detectados durante a avaliação de comorbidades, podendo ser reversíveis. A doença pode progredir rapidamente e desencadear insuficiência renal em poucos meses; no entanto, na maior parte dos casos a doença evolui lentamente ao longo de décadas (KALANTAR-ZADEH *et al*, 2021; LEVEY & CORESH, 2012). Considerando o papel central da TFG na fisiopatologia e complicações da DRC, a doença é classificada em cinco estágios: estágio 1, TFG > 90 ml/min/1,73m^2; estágio 2, TFG 60–89 ml/min/1,73m^2; estágio 3, TFG 30–59 ml/min/1,73m^2; estágio 4, TFG 15–29 ml/min/1,73m^2; estágio 5, TFG < 15 ml/min/1,73m^2. O estágio 5 da DRC ou doença renal terminal (DRT) consiste da falência renal, que além de reduzida TRG também é caracterizada pela necessidade de terapia renal substitutiva (hemodiálise ou transplante renal) (LEVEY & CORESH, 2012).

A DRC apresenta etiologia complexa e multifatorial, o que dificulta o seu diagnóstico. Em países desenvolvidos, a DRC é geralmente associada ao envelhecimento, diabetes, obesidade e doenças cardiovasculares, especialmente hipertensão arterial sistêmica. Em países em desenvolvimento, doenças tubulointersticiais resultantes de infecções e exposição a drogas e toxinas também constituem causas frequentes de DRC (KALANTAR-ZADEH *et al*, 2021; LEVEY & CORESH, 2012). A DRC tem recebido cada vez mais atenção da comunidade

científica internacional, uma vez que o aumento na sua prevalência vem sendo demonstrado em estudos recentes. Foi particularmente significante o estudo do *National Health and Nutrition Examination Survey* (NHANES), conduzido entre 1999 e 2004, que envolveu uma amostra expressiva (n = 13.233) da população de adultos não institucionalizados dos EUA, com 20 anos de idade ou mais. Nesse estudo, a prevalência da DRC foi determinada com base na presença de albuminúria persistente (> 30 mg/g) e diminuição da TFG estimada usando a equação abreviada do estudo *Modification of Diet in Renal Disease* (MDRD), reexpressa para creatinina sérica padrão (LEVEY & CORESH, 2012). Essa análise revelou que aproximadamente 13% da população adulta nos EUA apresenta algum grau de DRC, variando entre os estágios 1 a 4 (CDC, 2007). Nesse mesmo país, os custos diretos com o tratamento de pacientes com DRC em estágio terminal supera 23 bilhões de dólares ao ano. Além disso, no ano de 2008 o custo médio anual por paciente em sessões de hemodiálise foi de 77.506 mil dólares, 57.639 mil para diálise peritoneal e 26.668 mil para transplante de rim. Devido ao elevado custo, o tratamento da DRC apresenta disponibilidade limitada em várias regiões do mundo, de modo que muitos pacientes com DRT morrem sem tratamento (LEVEY & CORESH, 2012).

No Brasil, estudos epidemiológicos abrangentes sobre a DRC que empregam a nova definição da doença ainda não foram realizados. Entretanto, um estudo sobre terapia renal substitutiva (TRS) baseado em dados coletados em janeiro de 2009 identificou 77.589 pacientes em hemodiálise no Brasil e que a prevalência e a incidência de DRT correspondiam a cerca de 410 e 144 casos por milhão na população, respectivamente (SESSO *et al*., 2009). Extrapolando-se esses resultados para a população adulta brasileira, sugere-se que cerca de 2,9 milhões de brasileiros teriam um terço ou menos da TFG dos indivíduos normais (FERNANDES *et al*., 2010). No Brasil, a população em diálise vem aumentando progressivamente nos últimos anos: 42.695 no ano 2000, 92.091em 2010, 91.314 em 2011, e

97.586 em 2012. Em relação a 2010, houve um aumento de 3% ao ano no número de doentes renais crônicos em hemodiálise, sendo que mais da metade desses pacientes encontrava-se na região Sudeste. Os dados ilustram que a ocorrência de DRC encontra-se em ascensão, constituindo um importante problema de saúde pública que requer medidas urgentes para limitar esse crescimento (SESSO, 2012).

1.2 Doença renal crônica e alterações cardiovasculares

Estima-se que pacientes com DRT em diálise apresentam taxa de mortalidade superior a 20% ao ano, com mais da metade dos óbitos relacionados a doenças cardiovasculares (LEVEY & CORESH, 2012). Existem evidências de que cerca de 40% dos pacientes que iniciam o tratamento de diálise apresentam doença da artéria coronária, e 85% destes pacientes possuem estrutura e/ou função anormal do ventrículo esquerdo (SCHIFFRIN *et al*., 2007). Estimativas indicam que pacientes com DRT apresentam 15 a 30 vezes mais risco de mortalidade por eventos cardiovasculares em relação à população em geral. Esse risco é ainda 500 vezes maior em indivíduos adultos jovens (25 a 34 anos) com DRT comparados a indivíduos de mesma faixa etária na população geral (LEVEY & CORESH, 2012). Pacientes com DRT em hemodiálise (HD) podem ficar expostos a fatores cardiovasculares que aumentam os riscos de morbidade e mortalidade. Importantes fatores de risco incluem hipertensão arterial sistêmica, retenção de líquidos e gradiente de sódio medido como o ganho de peso interdialítico (KEEN & GOTCH, 2007; LEVEY & CORESH, 2012).

Os mecanismos pelos quais a disfunção renal pode levar a doença cardiovascular são múltiplos. Tanto fatores de risco tradicionais como não tradicionais estão implicados na patogênese da doença cardiovascular nos indivíduos com DRC. Os fatores de risco tradicionais principais são hipertensão arterial, diabetes, hiperuricemia e dislipidemia (principalmente elevação de triglicérides e diminuição de HDL-colesterol, secundários a

resistência periférica à ação da insulina causada pela própria toxicidade urêmica) (LONGENECKER *et al*., 2002; SCHIFFRIN *et al*., 2007). Dentre os fatores de risco não tradicionais destacam-se variáveis avaliadas rotineiramente para o acompanhamento clínico dos pacientes com DRC, tais como hiperparatireoidismo e alterações do metabolismo dos íons divalentes, anemia, sobrecarga hidrossalina; bem como variáveis não rotineiramente avaliadas na prática clínica como hiper-homocisteinemia, aumento do estresse oxidativo, disfunção endotelial, elevações das apolipoproteínas, acúmulo de dimetil arginina assimétrica e de mediadores pró-inflamatórios (LONGENECKER *et al*., 2002).

1.3 Doença renal crônica e inflamação sistêmica

A DRC é uma síndrome complexa associada a numerosas comorbidades, incluindo uma a síndrome urêmica-inflamatória crônica (COBO *et al*., 2018; JOFRÉ *et al*., 2006). Nessa síndrome, o acúmulo de toxinas urêmicas em resposta à perda da função exócrina renal, o contato do sangue com a membrana de diálise e a presença de contaminações nas fístulas vasculares ativam mecanismos pró-oxidantes e pró-inflamatórios com repercussões sistêmicas (COBO *et al*., 2018; JOFRÉ *et al*., 2006). Nesses processos, ocorre intensa produção e acúmulo de efetores imunológicos, como citocinas e quimiocinas, especialmente TNF, IL-1, IL-6, IFN, e MCP-1 (CASTILLO-RODRÍGUEZ *et al*., 2017; YAVUZ *et al*., 2005; PANICHI *et al*., 2008, 2012). Em conjunto, essas moléculas estabelecem um estado pró-inflamatório constante que aumenta o risco de morbimortalidade em pacientes com doença renal terminal em hemodiálise (PANICHI *et al*., 2008; STENVINKEL *et al*., 2016; ZIMMERMANN *et al*., 1999). Atualmente, a inflamação crônica tem sido diretamente associada ao desenvolvimento e progressão de doenças cardiovasculares em pacientes com doença renal terminal, especialmente calcificação vascular e aterosclerose (PANICHI *et al*., 2012; ZIMMERMANN *et al*., 1999). A relação entre os níveis circulantes de diferentes efetores imunológicos e a

patogênese das doenças cardiovasculares é complexa e ainda não está completamente elucidada para esses pacientes. No entanto, estudos prévios indicam que elevados níveis circulantes de moléculas como fator de necrose tumoral (TNF), interleucina 6 (IL-6) e proteína quimioatraente de monócitos do tipo 1 (MCP-1) estão associados ao maior comprometimento cardiovascular e elevado risco de mortalidade em pacientes sob tratamento hemodialítico (BARRETO *et al*., 2010; CASTILLO-RODRÍGUEZ *et al*., 2015; PANICHI *et al*., 2012).

Existe evidência de que diversas citocinas e quimiocinas podem ser úteis como marcadores do estado inflamatório sistêmico, da qualidade da diálise e da resposta ao tratamento hemodialítico (COBO *et al*., 2018; PANICHI *et al*., 2008, 2012). Além disso, essas moléculas são relevantes com indicadoras da gravidade da doença cardiovascular, de prognóstico, do risco de mortalidade por eventos cardiovasculares e por causas gerais em pacientes com doença renal crônica (NOWAK AND CHONCHOL, 2018; PANICHI *et al*., 2008, 2012). No entanto, a dosagem de citocinas e quimiocinas não é realizada na rotina clínica de acompanhamento do paciente em hemodiálise, especialmente em função do custo elevado e da necessidade de equipamentos, reagentes e recursos humanos especializados nas técnicas de análise dessas moléculas. Nesse sentido, existe grande dificuldade para utilizar as citocinas e as quimiocinas para avaliar o estado inflamatório desses pacientes, tornando-se relevante identificar parâmetros clínicos, nutricionais e bioquímicos obtidos na rotina clínica e que podem ser úteis como preditores dos níveis de citocinas e quimiocinas em pacientes sob tratamento hemodialítico. Assim, avaliar os tipos, os níveis e os fatores associados à distribuição de citocinas e quimiocinas pode ser relevante para estimar o estado inflamatório sistêmico nesses pacientes (NOWAK AND CHONCHOL, 2018; PANICHI *et al*., 2012). Entender a dinâmica de diferentes efetores imunológicos pode ser relevante para aprimorar os protocolos terapêuticos e atenuar a morbimortalidade em pacientes em hemodiálise,

especialmente considerando a evidência de que a inflamação crônica pode ser o principal determinante da fisiopatologia das doenças cardiovasculares em portadores e doença renal crônica (BARRETO *et al*., 2010; PANICHI *et al*., 2008).

2 OBJETIVOS

2.1 Objetivo geral

Avaliar a relação entre a dose de diálise, marcadores metabólicos séricos, e os níveis sistêmicos de citocinas e quimiocinas em pacientes com doença renal terminal em tratamento hemodialítico.

2.2 Objetivos específicos

a) Investigar a qualidade da diálise em pacientes com doença renal terminal;

b) Aferir a relação entre a qualidade de diálise e os níveis de marcadores bioquímicos séricos em pacientes em hemodiálise;

c) Determinar os níveis séricos de citocinas e quimiocinas pró-inflamatórias e anti-inflamatórias em pacientes em hemodiálise;

d) Avaliar a relação entre a qualidade de diálise e os níveis séricos de citocinas e quimiocinas pró-inflamatórias e anti-inflamatórias em pacientes em hemodiálise;

e) Caracterizar o potencial preditivo do Kt/V e de marcadores bioquímicos para predizer a inflamação sistêmica indicada pelos níveis séricos de citocinas e quimiocinas pró-inflamatórias e anti-inflamatórias em pacientes em hemodiálise.

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4 CAPÍTULO 1 - Manuscrito formatado e publicado no periódico *International Immunopharmacology* (Anexo 1)

Cytokines and chemokines systemic levels are related to dialysis adequacy and creatinine clearance in patients with end-stage renal disease undergoing hemodialysis

4.1 Introduction

Chronic kidney disease (CKD) is an impacting public health problem worldwide, causing high social and economic costs to the health system (Hill *et al*., 2016). Epidemiological evidences indicated a global prevalence of 753 million people with CKD (i.e. 417 million females and 336 million males) in 2016 (Bikbov *et al*., 2018). This disease is associated with high morbidity and mortality, especially in poor countries due to the limited access to specialized and more expensive treatments, such as renal replacement therapies (Ferraz *et al*., 2017). Thus, renal failure has been responsible by over 1 million deaths/year in developing countries (Couser *et al*., 2011; Wang *et al*., 2021). CKD etiology is complex and often multifactorial. However, systemic arterial hypertension, diabetes mellitus, renal infections, and chronic immunomediated diseases are the main comorbidities associated with the high incidence, prevalence and severity of CKD worldwide (Cohen, 2020; Zou *et al*., 2020).

In more severe cases and in the absence of adequate treatment, the patient progresses to end-stage renal disease (ESRD), requiring renal replacement therapy (i.e., hemodialysis (HD) or transplantation) (De Rosa *et al*., 2017). The mortality rates in ESRD patients is 2.5 to 10 times higher compared to general population, and is mainly associated to cardiovascular diseases triggered by chronic systemic inflammatory processes (Rios *et al*., 2017). Currently, the pathogenesis of the pro-inflammatory status in HD patients is not completely understood

(Cohen and Narayanan, 2019). However, the accumulation of uremic toxins (i.e., urea, indoxyl sulphate, end products of advanced glycation and calcioprotein particles), infections of venous catheters, bio-incompatible of dialysis membranes, immune dysfunction, gut dysbiosis, and malnutrition contribute to the chronic pro-inflammatory uremic phenotype that aggravates the morbimortality risk in HD patients (Vianna *et al*., 2011; Sharif *et al*., 2015; Cobo *et al*., 2018; Kaysen, 2021).

Among the heterogeneous group of uremic toxins, protein-bound solutes and the middle molecules (0.5-60 kD) (15,16) are poorly filtered during dialysis, representing a major group of pro-inflammatory molecules in ESRD (13,14). In this group, immunological effectors such as the cytokines tumor necrosis factor (TNF), interleukin (IL-) 2 and 6, as well as the chemokines CCL-2 and CXCL8 exhibits a marked retention and contributes to adverse clinical outcomes in HD patients (de Lima *et al*., 2013; Castillo-Rodríguez *et al*., 2017; Vanholder *et al*., 2018). As most dialysis techniques used in clinical routine are ineffective in ensuring an adequate clearance of these uremic toxins, the cumulative metabolic stress increases the inflammatory allostatic load, making the management of HD patients' a challenging task (Castillo-Rodríguez *et al*., 2017).

Currently, the refinement of the uremic control from dialysis optimization has indicated that circulating levels of middle molecules exerts a direct impact on the clinical outcomes of HD patients (Steyaert *et al*., 2019). Accordingly, these molecules have been consistently used as biomarkers in ESRD, exhibiting a determined predictive value associated to the clinical condition/disease severity, treatment efficacy, and patients' prognosis (Akchurin and Kaskel, 2015). Despite the diversity of laboratory measurements available, the net urea clearance rate remains the best studied measure to dialysis adequacy (Bharati and Jha, 2020). Thus, monitoring urea clearance using the Kt/V method (21) is often used to access dialysis dose (i.e., adequacy and effectiveness) (Steyaert *et al*., 2019), which is closed

correlated to morbimortality rates in HD patients (Mandolfo *et al*., 2000; Nemati *et al*., 2016; Steyaert *et al*., 2019). Although the adequacy of the dialysis dose by Kt/V represent a relevant strategy associated to uremic syndrome correction in ESRD patients (Vanholder et *et al*., 2018), this approach is essentially based the clearance of low molecular weight toxins. However, the relationship between Kt/V and middle uremic toxins is often overlooked.

Considering that the accumulation of middle inflammatory effectors represents an direct role in immune deficiency and clinical decay of HD patients, the present study investigated the relationship between dialysis adequacy, biochemical markers, cytokines and chemokines circulating levels in ESRD patients undergoing HD.

4.2 Patients and Methods

The study was conducted in accordance with the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee in Human Research (protocol 1.767.706). Forty-eight volunteers (21 women and 27 men) with ESRD undergoing hemodialysis were included in the present study, and all participants signed the Informed Consent. Patients' selection was based on well-defined exclusion criteria: i) hemodynamic instability, ii) history of renal transplantation, iii) change in dialysis modality during the last 3 months, iv) newly implanted catheters, v) minimum 6-month HD time, vi) neoplastic disease, vi) cognitive deficit evaluated by the Mini Mental State Examination, vii) refuse to participate in the study (Silva *et al*., 2019). Four kidney-transplant recipients (2 women and 2 men) were excluded, and forty-four patients (19 women and 25 men) were investigated. All patients received regular HD sessions for 3-4h, three times a week. Blood flow ranged from 300-450 mL/min with a dialysate stream at a constant rate (500 mL/min). Dialysis was based on low flow polysulfone membranes and high flux polysulfone membranes with bicarbonate-buffered dialysate.

4.2.1 Clinical data record

All clinical data were collected the same nephrologist (R.E.S.) responsible for clinical follow-up of the patients in the hemodialysis center. Data were recorded during the second weekly HD session. Characteristics such as gender, age, body mass index, time on hemodialysis, comorbidities, smoking, alcohol intake, and physical exercise were collected from the medical record and confirmed with all patients, when appropriated. The dialysis dose was calculated from the Kt/V method as follows: Kt/V = - ln (R - 0.008 × t) + (4 - 3.5 × R) 0.55 × UF / V; where R is U*pre*/U*post*, t is the duration of the session in hours, - ln is the natural logarithm negative, UF is the weight loss in kilograms and V is the volume of urea distribution in liters (Lowrie and Teehan, 1983). Nutritional assessment was conducted by the same nutritionist (P.B.I.J.) of the hemodialysis center, and was based on body mass index (BMI = body mass [kg]/ height [m^2]) and the global objective assessment (GOA) for HD patients (Riella and Martins, 2001; Silva *et al*., 2019).

4.2.2 Biochemical data record

Blood samples were collected using Vacutainer® tubes (Gel SST II Advance, Becton Dickinson, San Jose, CA, USA) immediately before HD session to avoid dialysis influence on blood parameters (Silva *et al*., 2019). Blood samples were centrifuged at 4ºC for 15 min (3800 ×*g*), and the serum was collected. Hemoglobin, hematocrit, leucocytes and platelets were quantified in a hematological analyzer by using high-grade human reagents (Sysmex, XE-2100, Sao Paulo, SP, Brazil). Urea, creatinine, blood glucose, potassium, calcium, phosphorus, iron, ferritin, total protein, albumin, alkaline phosphatase (ALP), glutamic-pyruvic transaminase (GPT), and parathyroid hormone (PTH) were analyzed by spectrophotometry using commercial diagnostic colorimetric kits (Invitro, Itabira, MG, Brazil). Total iron-binding capacity was measured by spectrophotometry using commercial

kits (Labtest, Lagoa Santa, MG, Brazil). Transferrin saturation index (TSI, %) was calculated as follows: TSI = (serum iron level × 100%) / total iron-binding capacity (Beilby *et al*., 1992).

4.2.3 Cytokines and chemokines immunoassay

The cytokines interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-17 (IL-17), interferon gamma (IFN-γ), and the chemokines C-C motif chemokine ligand 2 (CCL-2/MCP-1) and 5 (CCL-5/RANTES), C-X-C motif chemokine ligand 8 (CXCL-8/IP-10) and 10 (CXCL-10/IP-10) were quantified in the serum by flow cytometry bead array (CBA). Serum from 44 healthy volunteers with same sex distribution (19 women and 25 men), similar age (54.77 ± 15.09) and body mass index (23.89 ± 7.11) was collected to estimate cut-off points for cytokines and chemokines, allowing the assessment of the inflammatory status of HD patients. Commercial kits for cytokines (Human Th1/Th2/Th17 Kit) and chemokines (Human chemokines Kit) were used following the manufacturer's instructions (BD Biosciences, San Diego, CA, USA). All molecules were quantified in the FACSVerse flow cytometer (BD Biosciences, San Diego, CA, USA). The results were obtained from the FCAP 3.0 software. Standard curves were prepared using recombinant cytokines and chemokines at 20 to 5000 pg/mL. The lower limit of CBA-based cytokines detection was 2.6 to 18.9 pg/mL and CBA-based chemokines detection was 2 to 2.8 pg/mL.

4.2.4 Statistical analysis

Statistical procedure was based on data stratified by gender (women × men) and Kt/V ($<1.2 \times \geq 1.2$ values). Categorical variables were represented absolute values and frequencies, and were compared using the Pearson’s chi-squared and Fisher’s exact tests. For continuous variables, data distribution was verified by D'Agostino-Pearson test and expressed as mean and standard deviation. Continuous data were compared using Mann–Whitney U test

(nonparametric data) *and* Student's *t-test* (parametric data). The association between Kt/V, creatinine and urea with cytokines and chemokines serum levels was analyzed by Pearson correlation coefficient and multiple linear regression. All tests were based on 95% reliability, and results with $P<0.05$ indicated statistical difference.

4.3 Results

As indicated in table 1, our sample was mainly formed by patients (56.82% men and 43.18% women) ranging from 19 to 65 years of age, with similar body mass and BMI. All patients exhibited systemic arterial hypertension, in some cases associated to diabetes mellitus (13.64%) and heart disease (27.27%). The frequency of smoking and family history of kidney disease were low (20.46% each), and alcohol intake neither physical exercise practice was not reported. The mean time in HD was 4.09 ± 2.77 years, and most patients (63.34) presented adequate dialysis dose ($Kt/V\geq1.2$). Except for comorbidities ($P<0.05$), gender did not influence the general characteristics of the investigated sample ($P>0.05$), which exhibited marked similarity.

Table 1. General characteristics of all patients with end-stage renal disease undergoing hemodialysis stratified by sex.

Variables	Total (n= 44)	Women (n= 19)	Men (n= 25)	*P value**
Age (years), mean ± S.D.	53.77 ± 16.62	55.00 ± 17.34	52.84 ± 16.36	*0.674*[(a)]
Body mass (kg), mean ± S.D.	67.77 ± 15.51	63.47 ± 15.00	71.06 ± 15.38	*0.109*[(a)]
Body mass index, mean ± S.D.	25.60 ± 5.70	26.21 ± 6.43	25.13 ± 5.16	*0.540*[(a)]
Comorbidities, n (%)				
SAH	26 (59.09)	8 (42.11)	18 (72.00)	
DM + SAH	6 (13.64)	2 (10.53)	4 (16.00)	***0.033***[(b)]
SAH+ CHF	12 (27.27)	9 (47.37)	3 (12.00)	
Smoking, n (%)				
Yes	9 (20.46)	2 (10.53)	7 (28.00)	*0.260*[(b)]
No	35 (79.54)	17 (89.47)	18 (72.00)	
Alcohol intake, n (%)				
Yes	(0.00)	0 (0.00)	0 (0.00)	*1.00*[(b)]
No	44 (100)	19 (100)	25 (100)	
Physical exercise, n (%)				
Yes	(0.00)	0 (0.00)	0 (0.00)	*1.00*[(b)]
No	44 (100)	19 (100)	25 (100)	
Family history of kidney disease, n (%)				
Yes	9 (20.46)	3 (15.79)	6 (24.00)	*0.709*[(b)]
No	35 (79.54)	16 (84.21)	19 (76.00)	
Time in hemodialysis (years), mean ± S.D.	4.26 ± 2.80	4.56 ± 2.83	4.04 ± 2.85	*0.400*[(a)]
Kt/V, mean ± S.D.	1.37 ± 0.28	1.42 ± 0.34	1.34 ± 0.23	*0.627*[(a)]
Kt/V				
< 1.2	16 (36.36)	8 (42.11)	8 (32)	*0.540*[(b)]
≥ 1.2	28 (63.34)	11 (57.89)	17 (68)	
GOA, n (%)				
Appropriate	2 (4.55)	2 (110.5)	0 (0.0)	*0.087*(b)
Mild/moderate	42 (95.45)	17 (89.5)	25 (100.0)	

DM, diabetes mellitus; SAH, systemic arterial hypertension; CHF, congestive heart failure, GOA, global objective nutritional assessment. P values represent the result of [(a)] Student's t test or Mann-Whitney U test for continuous variables, and [(b)] Pearson's chi-squared test or Fisher's exact test for

categorical variables. **P* values in bold indicates significant difference among the groups stratified by sex ($P \leq 0.05$).

When the sample was stratified according dialysis adequacy measured by Kt/V method, only patients with Kt/V<1.2 presented a higher body mass compared to patients with adequate dialysis dose (P<0.05). However, age, comorbidities, smoking, alcohol intake, physical exercise, Family history of kidney disease, and time hemodialysis were similar in both groups (P>0.05). As BMI and nutritional status assessed by Global Objective Assessment (GOA) was similar in both Kt/V groups (P>0.05), the interference of these parameters on biochemical and immunological results were irrelevant (Table 2).

Table 2. General characteristics of all patients with end-stage renal disease undergoing hemodialysis stratified by dialysis adequacy measured according Kt/V method.

Variables	Kt/V < 1.2 (n= 16)	Kt/V ≥ 1.2 (n= 28)	*P value*
Age (years), mean ± S.D.			
	53.13 ± 14.65	54.14 ± 17.89	*0.848*[(a)]
Body mass (kg), mean ± S.D.			
	73.56 ± 12.12	64.48 ± 16.45	***0.031***[(a)]
Body mass index, mean ± S.D.			
	27.33 ± 4.26	24.60 ± 6.23	*0.128*[(a)]
Comorbidities, n (%)			
SAH	9 (56.25)	18 (64.29)	
DM + SAH	2 (12.5)	3 (10.71)	*0.868*[(b)]
HAS + CHF	5 (31.25)	7 (25.00)	
Smoking, n (%)			
Yes	3 (18.75)	6 (21.43)	
No	13 (81.25)	22 (78.57)	*1.00*[(b)]
Alcohol intake, n (%)			
Yes	0 (0.0)	0 (0.0)	*1.00*[(b)]
No	16 (100.0)	28 (100.0)	
Physical exercise, n (%)			
Yes	0 (0.0)	0 (0.0)	*1.00*[(b)]
No	16 (100.0)	28 (100.0)	
Family history of kidney disease, n (%)			
Yes	3 (18.75)	6 (21.43)	*1.00*[(b)]
No	13 (81.25)	22 (78.57)	
Time hemodialysis (years), mean ± S.D.			
	3.25 ± 1.29	4.82 ± 3.24	*0.121*[(a)]
Kt/V, mean ± S.D.			
	1.10 ± 0.09	1.52 ± 0.24	***<0.001***[(a)]
Global objective assessment, n (%)			
Appropriate nutrition	2 (12.5)	0 (0.0)	*0.126*[(b)]
Mild/moderate malnutrition	14 (87.5)	28 (100.0)	

DM, diabetes mellitus; SAH, systemic arterial hypertension; CHF, congestive heart failure; GOA: global objective assessment. P values represent the result of [(a)] Student's t test or Mann-Whitney U test for continuous variables, and [(b)] Pearson's chi-squared test or Fisher's exact test for categorical variables. **P* values in bold indicates significant difference among the groups stratified by sex ($P \leq 0.05$).

As reported in table 3, biochemical analysis indicated that women in HD presented reduced hemoglobin, pre- and post-HD urea compared to men (P<0.05). However, most parameters such as hematocrit, leucocytes, platelets, creatinine, blood glucose, potassium, calcium, phosphorus, iron, ferritin, total protein, albumin, ALP, GPT, TSI, and GPT were similar according gender stratification (P>0.05).

Table 3. Blood biochemical parameters all patients with end-stage renal disease undergoing hemodialysis stratified by sex.

Variables	Total (n= 44)	Women (n= 19)	Men (n= 25)	*P value**
Hemoglobin (g/dL)	10.9 ± 2.2	10.1 ± 2.1	11.5 ± 2.2	***0.036***$^{(a)}$
Hematocrit (%)	34.1 ± 6.5	32.3 ± 6.0	35.5 ± 6.7	*0.115*$^{(a)}$
Leucocytes × 10^2/µl	67.3 ± 18.3	68.1 ± 20.8	66.7 ± 16.6	*0.811*$^{(a)}$
Platelets × 10^4/µl	19.7 ± 6.9	20.1 ± 8.3	19.5 ± 5.7	*0.761*$^{(a)}$
Pre-HD urea (mg/dL)	106.36 ± 32.4	94.7 ± 32.9	115.2 ± 29.8	***0.037***$^{(a)}$
Post-HD urea (mg/dL)	36.5 ± 23.4	25.8 ± 17.2	44.6 ± 24.5	***0.007***$^{(a)}$
Creatinine (mg/dL)	13.2 ± 23.1	17.7 ± 35.3	10.5 ± 2.6	*0.100*$^{(b)}$
B. glucose (mg/dL)	145.1 ± 71.2	123.1 ± 44.7	161.8 ± 83.2	*0.265*$^{(b)}$
Iron (mg/dL)	60.9 ± 30.5	58.2 ± 24.0	63.1 ± 34.9	*0.605*$^{(a)}$
Ferritin (ng/dL)	309.6 ± 317.1	286.4 ± 281.1	327.1 ± 346.6	*0.906*$^{(b)}$
T. Protein (mg/dL)	7.2 ± 0.8	7.1 ± 0.8	7.27 ± 0.8	*0.395*$^{(a)}$
Albumin (g/dL)	3.9 ± 0.5	3.7 ± 0.5	3.9 ± 0.4	*0.166*$^{(b)}$
ALP (IU/L)	271.0 ± 342.7	348.4 ± 466.4	212.3 ± 197.3	*0.303*$^{(b)}$
GPT (IU/L)	17.5 ± 7.9	15.7 ± 6.8	18.9 ± 8.5	*0.169*$^{(b)}$
TSI (%)	23.1 ± 10.2	21.2 ± 7.9	24.5 ± 11.7	*0.284*$^{(a)}$
PTH (pg/mL)	621.8 ± 540.1	673.1 ± 686.5	582.8 ± 406.8	*0.804*$^{(b)}$
Kt/V	1.37 ± 0.28	1.42 ± 0.34	1.33 ± 0.23	*0.627*$^{(b)}$

B. glucose, blood glucose; GPT, glutamic-pyruvic transaminase; T. Protein, total protein; ALP, Alkaline phosphatase; TSI, transferrin saturation index; PTH, parathyroid hormone. *P values in bold indicates significant difference (P≤0.05) among the groups stratified by sex and obtained from $^{(a)}$Student's t test or $^{(b)}$Mann-Whitney U test, according data distribution.

As indicated in table 4, when the sample was stratified according dialysis adequacy measured by Kt/V method, patients with Kt/V < 1.2 presented increased leucocytes levels, post-HD urea and creatinine compared to patients with adequate dialysis dose (P<0.05). All the other biochemical parameters investigated were similar in both Kt/V groups (P>0.05).

Table 4. Blood biochemical parameters of patients with end-stage renal disease undergoing hemodialysis stratified by dialysis adequacy measured according Kt/V method.

Variables	Kt/V < 1.2 (n= 16)	Kt/V ≥ 1.2 (n = 28)	*P value**
Hemoglobin (g/dL)	10.51 ± 1.65	11.08 ± 2.50	*0.420*[(a)]
Hematocrit (%)	33.30 ± 4.67	34.57 ± 7.41	*0.541*[(a)]
Leucocytes × 10^2/µl	79.58 ± 18.74	60.32 ± 14.12	***<0.001***[(a)]
Platelets × 10^4/µl	20.22 ± 8.49	19.46 ± 5.92	*0.730*[(a)]
Pre-HD urea (mg/dL)	112.43 ± 42.16	102.89 ± 55.54	*0.354*[(a)]
Post-HD urea (mg/dL)	47.07 ± 60.65	31.85 ± 16.54	***0.004***[(a)]
Creatinine (mg/dL)	19.94 ± 8.01	9.29 ± 2.42	***0.023***[(b)]
B. glucose (mg/dL)	146.5 ± 79.45	144.32 ± 67.64	*0.884*[(b)]
Iron (mg/dL)	18.68 ± 8.26	16.89 ± 7.70	*0.373*[(a)]
Ferritin (ng/dL)	6.918 ± 0.88	7.32 ± 0.74	*0.110*[(b)]
T. Protein (mg/dL)	3.69 ± 0.52	3.96 ± 0.40	*0.061*[(a)]
Albumin (g/dL)	266.62 ± 260.92	273.57 ± 386.22	*0.903*[(a)]
ALP (IU/L)	62.75 ± 33.90	59.96 ± 28.94	*0.774*[(b)]
GPT (IU/L)	207.90 ± 235.40	367.63 ± 345.95	*0.095*[(b)]
TSI (%)	21.65 ± 9.87	23.89 ± 10.50	*0.491*[(a)]
PTH (pg/mL)	635.89 ± 556.30	613.79 ± 540.75	*0.874*[(b)]

B. glucose, blood glucose; GPT, glutamic-pyruvic transaminase; T. Protein, total protein; ALP, Alkaline phosphatase; TSI, transferrin saturation index; PTH, parathyroid hormone. *P values in bold indicates significant difference (P≤0.05) among the groups stratified by sex and obtained from [(a)]Student's t test or [(b)]Mann-Whitney U test, according data distribution.

As shown in figure 1, patients undergoing HD exhibited higher cytokines (IFN-γ, IL-4, IL-10, and IL-17) and chemokines (CCL-2/MCP-1, CCL-5/RANTES, CXCL-8/IL-8, and CXCL-10/IP-10) circulating levels compared to health control volunteers (P<0.05).

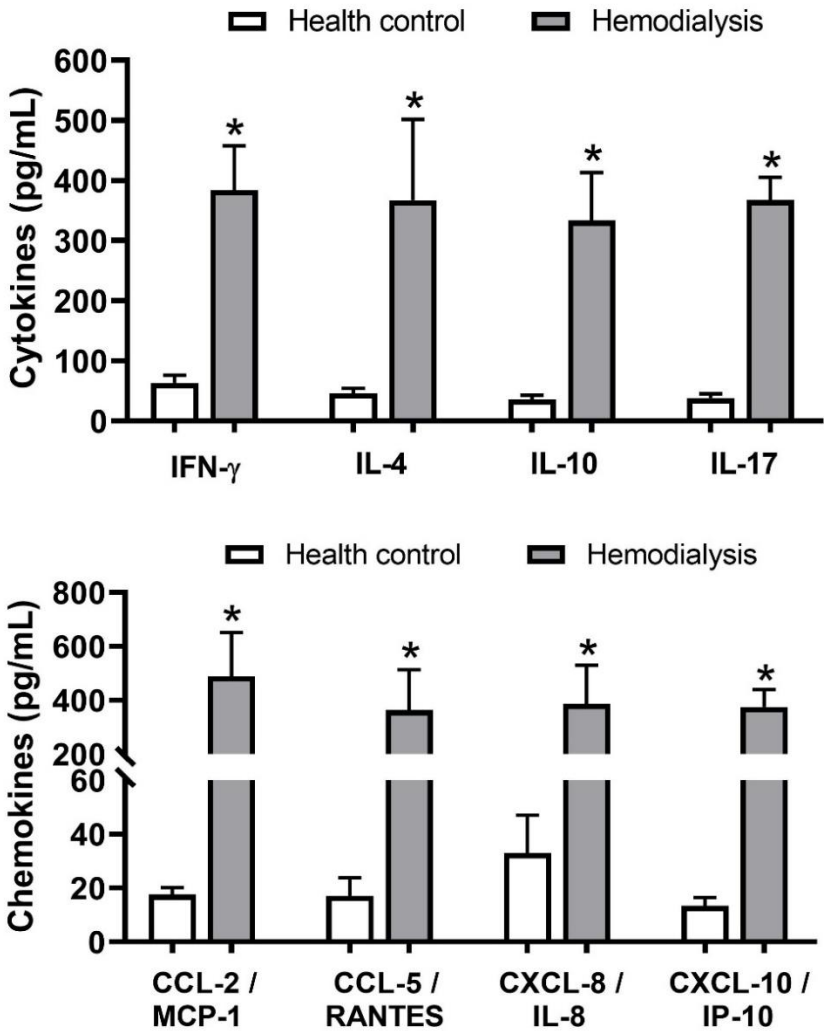

Fig. 1. Cytokines and chemokines serum levels in health control volunteers and patients with end-stage renal disease undergoing hemodialysis. * The symbol indicates significant difference (P≤0.05) among the groups from Student's t test.

Table 5 present the results obtained from cytokines and chemokines immunoassay. Patients with inadequate dialysis dose (Kt/V < 1.2) presented increased IFN-γ, CCL-2/MCP-1, CCL-5/RANTES, CXCL-8/IL-8, and CXCL-10/IP-10; as well as reduced IL-4 and IL-10

serum levels compared to patients with adequate dialysis dose (P<0.05). Only IL-17 circulating levels were similar in both Kt/V groups.

Table 5. Comparison between cytokines and chemokines serum levels in patients with end-stage renal disease undergoing hemodialysis stratified by dialysis adequacy measured according Kt/V method.

Variables	Kt/V < 1.2 (n= 16)	Kt/V ≥ 1.2 (n = 28)	*P value*
Cytokines (pg/mL)			
IFN-γ	443.16 ± 29.43	350.47 ± 69.85	***<0.001***
IL-4	204.17 ± 39.21	460.69 ± 54.52	***<0.001***
IL-10	292.46 ± 21.72	374.16 ± 79.47	***<0.001***
IL-17	380.26 ± 41.18	360.77 ± 34.19	*0.099*
Chemokines (pg/mL)			
CCL-2/MCP-1	646.89 ± 45.94	404.41 ± 76.56	***<0.001***
CCL-5/RANTES	510.23 ± 94.88	300.59 ± 68.81	***<0.001***
CXCL-8/IL-8	531.90 ± 96.59	315.33 ± 77.97	***<0.001***
CXCL-10/IP-10	440.19 ± 39.63	338.41 ± 43.05	***<0.001***

*P values in bold indicates significant difference (P≤0.05) among the groups stratified by sex and obtained from Student's t test or Mann-Whitney U test, according data distribution.

In patients stratified by Kt/V, a negative and significant correlation was observed between Kt/V and CXCL-10/IP-10 serum levels (Kt/V<1.2, R=-0.522 *vs.* Kt/V≥1.2, R= -0.612). This result was not influenced by dialysis adequacy. No significant correlation was detected between Kt/V and the other cytokines and chemokines investigated (Table 6).

Table 6. Correlation between dialysis adequacy measured by Kt/V method, cytokine and chemokine serum levels in patients with end-stage renal disease undergoing hemodialysis.

Variables	Kt/V < 1.2 (n= 16)		Kt/V ≥ 1.2 (n= 28)	
	Coefficient (R)	P value	Coefficient (R)	P value*
Cytokines (pg/mL)				
IFN-γ	0.123	*0.651*	-0.305	*0.114*
IL-4	-0.413	*0.112*	0.199	*0.311*
IL-10	-0.334	*0.207*	0.048	*0.808*
IL-17	-0.451	*0.079*	-0.168	*0.392*
Chemokines (pg/mL)				
CCL-2/MCP-1	-0.169	*0.532*	-0.302	*0.118*
CCL-5/RANTES	-0.434	*0.093*	-0.197	*0.314*
CXCL-8/IL-8	-0.351	*0.182*	-0.342	*0.075*
CXCL-10/IP-10	-0.522	***<0.001***	-0.612	***<0.001***

*P values in bold indicate significant correlation (Pearson correlation test) of Kt/V with cytokine and chemokines serum levels ($P \leq 0.05$).

As indicated in table 7, multiple linear regression reinforced the correlation between Kt/V and CXCL-10/IP-10 serum levels in both Kt/V groups. In our sample, 27% and 37% of CXCL-10/IP-10 variability were explained by Kt/V levels in the groups with Kt/V < 1.2 and Kt/V ≥ 1.2, respectively.

Table 7. Multiple linear regression model with cytokines and chemokines as dependent variables according dialysis adequacy measured by Kt/V method.

Variables	Kt/V < 1.2 (n= 16)			Kt/V ≥ 1.2 (n= 28)		
	β	R^2	*P*	*β*	R^2	*P*
Kt/V × Cytokine (pg/mL)						
IFN-γ	0.02360	0.015	*0.650*	-0.01127	0.093	*0.114*
IL-4	-0.006185	0.133	*0.181*	0.02218	0.039	*0.310*
IL-10	-0.009500	0.170	*0.111*	0.06307	0.002	*0.808*
IL-17	-0.006204	0.111	*0.206*	-0.04178	0.028	*0.391*
Kt/V × Chemokine (pg/mL)						
CCL-2/MCP-1	-0.004112	0.203	*0.079*	-0.009995	0.091	*0.117*
CCL-5/RANTES	-0.005325	0.028	*0.532*	-0.01770	0.038	*0.314*
CXCL-8/IL-8	-0.002034	0.188	*0.093*	-0.009019	0.116	*0.075*
CXCL-10/IP-10*	-0.004117	*0.273*	***<0.037***	-0.009129	0.374	***<0.001***

P values in bold indicate statistical significance for individual predictors in the regression models (P≤0.05). *Equations with significant result obtained from multiple linear regression analysis: For patients with Kt/V<1.2: CXCL-10 = 708.145 - (242,906 × Kt/V<1.2). For patients with Kt/V≥1.2: CXCL-10 = 517.557 - (117.691 × Kt/V≥1.2).

In patients with Kt/V < 1.2, a positive and significant correlation between creatinine with IFN-γ, CCL-2/MCP-1, and CXCL-10/IP-10 circulating levels (P<0.05). Inverse and significant correlation between creatinine and IL-10 was also identified in this group (P<0.05). In patients with Kt/V ≥ 1.2, IL-10 presented negative (P<0.05) and CXCL-10/IP-10 positive (P<0.05) correlation with creatinine levels (Table 8).

Table 8. Correlation between creatinine, cytokine and chemokine serum levels in patients with end-stage renal disease undergoing hemodialysis stratified by dialysis adequacy measured by Kt/V method.

Variables	Kt/V < 1.2 (n= 16)		Kt/V ≥ 1.2 (n= 28)	
	Coefficient (R)	P value	Coefficient (R)	P value*
Creatinine (mg/dL) × Cytokine (pg/mL)				
IFN-γ	0.682	***0.005***	0.071	*0.719*
IL-4	-0.225	*0.420*	0.170	*0.387*
IL-10	-0.608	***0.016***	-0.438	***0.019***
IL-17	0.339	*0.217*	0.304	*0.116*
Creatinine (mg/dL) × Chemokines (pg/mL)				
CCL-2/MCP-1	0.540	***0.037***	0.228	*0.244*
CCL-5/RANTES	0.280	*0.312*	0.219	*0.263*
CXCL-8/IL-8	0.319	*0.247*	0.101	*0.609*
CXCL-10/IP-10	0.591	***0.020***	0.108	***0.584***

*P values in bold indicate significant correlation (Pearson correlation test) of Kt/V witth cytokine and chemokines serum levels ($P \leq 0.05$).

As indicated in table 9, multiple linear regression corroborated the correlation between creatinine with IFN-γ, CCL-2/MCP-1, and CXCL-10/IP-10 circulating levels for patients with Kt/V < 1.2 (P<0.05). In this case, 47%, 37%, 29% and 35% of IFN-γ, IL-10, CCL-2/MCP-1 and CXCL-10/IP-10 variability were respectively explained by creatinine levels (P<0.05). In patients with Kt/V ≥ 1.2, creatinine levels were able to explain 19% IL-10 variability (P<0.05). Creatinine levels did not show a predictive value for the other cytokines and chemokines investigated (P>0.05).

Table 9. Multiple linear regression model with cytokines and chemokines as dependent variables according creatinine serum levels in patients with end-stage renal disease undergoing hemodialysis stratified by dialysis adequacy measured by Kt/V method.

Variables	Kt/V < 1.2 (n= 16)			Kt/V ≥ 1.2 (n= 28)		
	β	R^2	*P*	*β*	R^2	*P*
Creatinine (mg/dL) × Cytokine (pg/mL)						
IFN-γ	0.1703	0.465	***0.005***	0.4892	0.005	*0.719*
IL-4	-0.3767	0.050	*0.419*	0.2623	0.028	*0.387*
IL-10	-0.2404	0.369	***0.016***	-0.06973	0.192	***0.019***
IL-17	0.2636	0.114	*0.216*	0.2340	0.092	*0.116*
Creatinine (mg/dL) × Chemokines (pg/mL)						
CCL-2/MCP-1	0.1377	0.291	***0.037***	0.1342	0.051	*0.244*
CCL-5/RANTES	0.1210	0.078	*0.312*	0.1612	0.048	*0.262*
CXCL-8/IL-8	0.1049	0.101	*0.247*	0.3082	0.010	*0.608*
CXCL-10/IP-10*	0.1411	0.349	***0.020***	0.5216	0.011	*0.583*

P values in bold indicate statistical significance for individual predictors in the regression models (P≤0.05). *Equations with significant result obtained from multiple linear regression analysis: For patients with Kt/V<1.2: CXCL-10 = 910.449 - (426.298 × Kt/V<1.2). For patients with Kt/V≥1.2: CXCL-10 = 517.557 - (117.691 × Kt/V≥1.2).

4.4 Discussion

Considering that accumulation of middle uremic toxins, dialysis efficiency, and biochemical/metabolic status exert a marked impact on the clinical outcomes in ESRD patients (Hill *et al*., 2016; Nemati *et al*., 2016; Hekmat, 2020), this study investigated the relationship between dialysis adequacy, biochemical parameters, and the levels of pro- and anti-inflammatory effectors (cytokines and chemokines) in ESRD patients undergoing HD. When stratified by sex, our sample consisted of patients with similar clinimetric and biochemical characteristics. In addition, biochemical differences limited to leucocytes levels, *post-dialysis* blood *urea*, creatinine and calcium levels were identified after sample

stratification by Kt/V. However, marked divergences in cytokines and chemokines circulating levels were revealed from this stratification. Accordingly, Kt/V values were significantly correlated with CXCL-10/IP-10, but presented limited predictive value for this chemokine in patients exposed to adequate or inadequate dialysis dose. In addition, creatinine accumulation was directly correlated with IFN-γ, CCL-2/MCP-1 and CXCL-10/IP-10, but inversely correlated to IL-10 levels. Interestingly, creatinine exhibited predictive value for all these molecules when Kt/V < 1.2, which was limited to IL-10 for patients with adequate dialysis dose (Kt/V ≥ 1.2).

Considering general characteristics of all patients investigated, only comorbidities profile was different from sex stratification. These divergences were consistent with epidemiologic and demographic studies, indicating a higher ESRD prevalence and incidence in male than female patients (USRDS, 2014; Bikbov *et al*., 2018). In addition, SAH and DM were the main comorbidities identified, which are the most common etiologies of ESRD worldwide (29,30). As SAH and DM are potentially preventable and/or treatable diseases and acts as major risk factor for cardiovascular death in HD patients (Vos *et al*., 2016; Cohen, 2020; Zou *et al*., 2020), a significant impact on survival predictors has been attributed to adequate control of these comorbidities (Ghaderian *et al*., 2015; Miskulin *et al*., 2019). Parameters such as time in HD, body mass index, smoking, alcohol intake, physical exercise, and nutritional status can also interfere in the clinical condition and outcomes of HD patients (Cobo *et al*., 2016). Although these characteristics can be influenced by age and sex (Cobo *et al*., 2016), they were similar in female and male patients, reinforcing that all patients investigated received and equivalent exposition to dialysis, were sedentary, non-drinkers, exhibited a low frequency of smoking and a paired nutritional status.

When the same set of variables were analyzed from Kt/V stratification, only body mass was higher in patients exposed to inadequate dialysis dose. However, body mass index was

similar in both Kt/V strata. Kt/V is the most frequent method used to determine the required dialysis dose, in which values ≥ 1.2 are interpreted as efficient doses (Steyaert *et al*., 2019; Silva *et al*., 2019). Given that women and people with low weight could benefit from a higher Kt/V (Pérez-García *et al*., 2019), a register of 64.48 kg body mass (Kt/V ≥ 1.2) compared to 73.56 kg (Kt/V < 1.2) was also suggestive of an adequate dialysis dose in the upper stratum (mean Kt/V= 1.52 ± 0.24). Conversely, higher body mass may reflect a greater distribution of urea (Vanholder and Ringoir, 1992), reinforcing the cause of inadequate dialysis dose in the patients classified in the lower stratum (mean Kt/V= 1.10 ± 0.09).

Despite CKD guidelines do not consider specific clinical targets stratified by sex (Locatelli *et al*., 2008), standard biochemical monitoring of blood samples showed discrepancies in hemoglobin, pre- and post-HD urea levels in our patients. There is evidence that the erythropoietin response can be modulates by sex in HD patients, leaving women more likely to respond poorly to this hormone (Ifudu *et al*., 2001; Khankin *et al*., 2010). Accordingly, we registered lower hemoglobin levels in women than man, reinforcing sex specificity in cutoff values to define anemia in these patients (i.e., hemoglobin of 13.8 *vs.* 15.7 in women *vs.* man, respectively) (Palmer *et al*., 2014). Similarly, the urea clearance values based on pre- and post-HD concentrations were lower in women. Although urea kinetics is not directly determined by sex, it offer a metabolic indicator of urea biogenesis that is directly correlated to the protein catabolic rate (Silva *et al*., 2019; Hasegawa *et al*., 2020). There is evidence that the combination of nutritional and biochemical markers provide a more comprehensive estimation of the catabolic condition in ESRD patients (NKF, 2000; Silva *et al*., 2019). In this sense, anthropometric parameters and malnutrition status were similar in female and male patients, suggesting that the main distinction between the sexes may be linked to increased protein catabolism in men, which requires further investigation as it does not appear to be associated with the dialysis dose in this study.

Like urea levels, a marked creatinine accumulation was detected in patients exposed to inadequate dialysis dose. Creatinine kinetic is also consistently used as a marker of muscle protein catabolism in HD patients, which is profoundly influenced by dietary protein intake, nutritional status, hydration, and dialysis clearance (Colman *et al*., 2005; Patel *et al*., 2013). In our study, all patients received continuous nutritional monitoring, reducing the influence of inappropriate diets on the response to dialysis treatment. Thus, the dialysis dose may be a major factor for the creatinine variations identified between the Kt/V strata. Creatine integrates the group of protein bound uremic retention solutes, representing an important predictor of clinical outcomes in HD patients (Cobo *et al*., 2018). In this sense, high creatinine circulating levels are often associated with increased mortality risk in HD patients (Wang *et al*., 2017; Ilic *et al*., 2018). This relation is complex, and both urea and creatinine accumulation in patients with Kt/V < 1.2 is consistent with an increased uremic stress, which invariably predisposes to a prooxidant and proinflammatory microenvironment (Cohen *et al*., 2020; Rios *et al*., 2017; Syed-Ahmed and Narayanan, 2019). Interestingly, the worsening of uremic status may be linked to an increased leukocytes number in patients exposed to inadequate dialysis doses. However, the pathophysiology of the chronic inflammatory syndrome in these patients is complex, and seems to be especially related to retention of medium uremic toxins, notably cytokines and chemokines (Mihai *et al*., 2018; Vanholder *et al*., 2018).

As expected, HD patients exhibited increased cytokines and chemokines serum levels compared to health volunteers, corroborating the pro-inflammatory status associated to HD. Classically, the imbalance in the production of anti- and pro-inflammatory cytokines and the chronic pro-inflammatory condition typically observed in HD patients manifest from a complex interaction of uremic toxin accumulation, dialysis characteristics and comorbidities (Rios *et al*., 2017; Mihai *et al*., 2018; Silva *et al*., 2019). There is consistent evidence that

persistent inflammation is directly associated to the pathogenesis of cardiovascular disorders in HD patients, accounting for a relevant part of the increased mortality risk compared to the general population (Zou *et al*., 2020; Silverstein, 2009). Considering Kt/V stratification, a clear divergence in leucocytes distribution, cytokine and chemokine levels was also observed in HD patients. Thus, the inadequate dialysis dose was consistent with up-regulation of pro-inflammatory cytokines and chemokines, as well as down-regulation of anti-inflammatory cytokines (Castillo-Rodríguez *et al*., 2017; Rios *et al*., 2017). In this sense, an inadequate dialysis dose seems to offer additional risk for the development of the persistent systemic inflammation in HD patients, a characteristic potentially related to the increased mortality risk identified in patients with inadequate Kt/V (Castillo-Rodríguez *et al*., 2017; Milan Manani, 2016).

Despite the differences associated with the dialysis dose, only the CXCL-10/IP-10 chemokine showed a negative and significant correlation with Kt/V values, regardless the stratum considered. In addition, Kt/V exhibited a limited predictive value for CXCL-10/IP-10 circulating levels. Interestingly, positive and significant correlation between creatinine, IFN-γ, CCL-2/MCP-1 and CXCL-10/IP-10 were observed in patients with inadequate dialysis dose. In this group, creatinine and IL-10 also exhibited negative and significant correlation. These findings reinforce the proposition that classic markers used in clinical routine may be relevant to estimate the inflammatory status in HD patients. Previous studies have shown that the monitoring of inflammatory mediators has marked clinical and prognostic relevance (Akchurin and Kaskel, 2015; Nowak and Chonchol, 2018). Accordingly, several cytokines and chemokines orchestrate pathological processes linked to metabolic and cardiovascular deterioration in HD patients (Lam, 2009; Stancu *et al*., 2018). Furthermore, by indicating the severity of the cardiovascular condition, these molecules became useful as predictors of mortality in this population (Cozzolino *et al*., 2018; Carracedo *et al*., 2020).

Considering the biological relevance of the molecules with significant correlation, CCL2/MCP-1 is an important macrophage chemoattractant factor and a biomarker of vascular inflammation (de Oliveira Junior *et al*., 2020). CCL2/MCP-1 upregulation is often associated to atherosclerotic lesions in HD patients, whose atherogenic and thrombogenic properties are potentiated by CXCL8/IL-8. In turn, CXCL8/IL-8 is a neutrophil chemotactic factor overexpressed in inflamed tissues and organs (Russo *et al*., 2014), which exhibits predictive relevance for cardiovascular mortality in HD patients (Panichi *et al*., 2006). In addition, CCL-5/RANTES exerts chemoattractant effects on cells involved in innate immunity, especially monocytes, natural killer cells, basophils, eosinophils and T lymphocytes. This chemokine contributes to Th1 phenotype polarization, reinforcing IFN-γ upregulation associated with uremic toxins overload in ESRD patients undergoing HD (Krensky and Ahn, 2007). IFN-γ is a potent pro-inflammatory effector with atherogenic potential, which is potentially mediated by the direct role of this cytokine in trigger macrophages activation and stimulates nitric oxide biosynthesis (Carrero *et al*., 2008; Silverstein, 2009; Mihai *et al*., 2018). IFN-γ are also a CXCL-10/IP-10 inductor (Hägele *et al*., 2009), a chemokine pointed as a relevant marker of renal diseases severity (i.e. allograft dysfunction and lupus nephritis) (Gao *et al*., 2020). CXCL10/IP-10 is still chemoattractant for leukocytes and plays angiostatic effects, contributing to disease progression and a worsening prognosis in CKD patients (Gao *et al*., 2020).

Unlike IFN-γ, IL-17, CCL2, CCL-5, CXCL8 and CXCL-10, immunological effectors such as IL-4 and IL-10 are typical Th2 and Treg cytokines that exert potent anti-inflammatory effects (Chung and Lan, 2011; Kany *et al*., 2019), which are often upregulated in ESRD patients (Chung and Lan, 2011; Mai *et al*., 2020). However, uremic syndrome demands higher levels of these cytokines to achieve consistent anti-inflammatory effects in HD patients compared to healthy individuals (Girndt *et al*., 2003). Unfortunately, efficient production of

these anti-inflammatory molecules occurs in less than a third of patients with ESRD, impairing the control of uremic inflammatory syndrome (Cohen *et al*., 2010; Girndt *et al*., 2003). From this perspective, IL-4 and IL-10 are admitted as counter-regulatory mechanisms to attenuate uremia- and dialysis-induced immunological activation from Th1 pro-inflammatory effectors (Akchurin and Kaskel, 2015; Girndt *et al*., 2003). Thus, monitoring these molecules is potentially useful to assess the systemic levels of protective immune effectors in HD patients (Akchurin and Kaskel, 2015; Gu *et al*., 2020). As direct cytokines and chemokines detection is not always available in clinical routine, estimates based on dialysis dose and creatinine levels may increase understanding of the relationship between metabolic overload and inflammatory stress in patients with ESRD undergoing HD. Based on this understanding and the use of predictive tools, adjustments in therapeutic management can be rationally adopted to better control the uremic-inflammatory syndrome that increases the risk of morbidity and mortality in HD patients.

4.5 Conclusion

Taken together, our findings provided evidence that in addition to modulating uremic toxins levels, such as urea and creatinine, dialysis dose is associated with circulating levels of inflammatory mediators. Thus, high cytokines and chemokines circulating levels are consistent with the lower clearance of uremic toxins in patients with inadequate dialysis doses. In addition, Kt/V results were inversely correlated with CXCL-10/IP-10 levels, while creatinine levels were directly correlated with IFN-γ, CCL-2/MCP-1 and CXCL-10/IP-10 levels, and inversely correlated with IL-10 circulating levels. Despite these relationships, Kt/V and creatinine results exhibited limited predictive value for the investigated cytokines and chemokines. Thus, identifying more sensitive and specific predictors of uremic-

inflammatory overload remains a challenge to improve evaluation, therapeutic management and clinical outcomes in ESRD patients undergoing HD.

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5 DISCUSSÃO

A partir desse estudo clínico transversal, nós investigamos potenciais relações entre a dose de diálise, parâmetros bioquínicos quantificados na rotina de um centro de hemodiálise, e níveis de citocinas e quimiocinas circulantes em pacientes com doença renal terminal em tratamento hemodialítico. A casuística do presente estudo foi composta por homens e mulheres com características clínicas similares, indicando a homogeneidade de nossa amostra. Apenas o perfil de comorbidades diferiu após a estratificação dos pacientes de acordo com o sexo. Esse aspecto é relevante, uma vez que favorece a comparação entre os pacientes sem que o sexo atue como um elemento de confundimento nas análises e intepretações dos resultados. Consideranto o perfil de comorbidades, os nossos achados foram consistentes com evidências prévias, indicando que hipertensão arterial sistêmica e o diabetes mellitus representam as comorbidades mais frequentemente associadas à doença renal crônica terminal, as quais correspondem às etiologias mais comuns dessa doença em todo o mundo (VEJAKAMA *et al*., 2015; VOS *et al*., 2016). Esse é um importante achado, uma vez que ambas etiologias são condições que podem ser prevenidas ou atenuadas a partir de intervenções nutricionais e farmacológicas associadas à prática regular de exercícios físicos (MARONI & MITCH, 1997; JOHANSEN, 2007; LEVEY & CORESH, 2012; PONGRAC BARLOVIC *et al*., 2019).

A partir das evidências de que a hipertensão arterial sistêmica e o diabetes mellitus também agravam a condição clínica e atuam como fatores de risco para mortalidade em pacientes com doença renal crônica, o tratamento dessas comorbidades é frequentemente incorporado nos protocolos de manejo clínico desses pacientes (VOS *et al*., 2016; COHEN, 2020; ZOU *et al*., 2020). Assim, o tratamento das comorbidades apresenta impacto positivo em melhorar o prognóstico, reduzindo o risco de mortalidade em pacientes com doença renal crônica (GHADERIAN *et al*., 2015; MISKULIN *et al*., 2019). Além da hipertensão arterial

sistêmica e do diabetes mellitus, o tempo em hemodiálise, a adiposidade corporal, o tabagismo, a ingestão de álcool, o estado nutricional e a prática de exercícios físicos também exerce importante impacto no estado geral e nos desfechos clínicos de pacientes em hemodiálise (COBO *et al*., 2016). Notadamente, essas características são influenciadas pelo sexo, idade e por aspectos socioculturais dos indivíduos (COBO *et al*., 2016). No entanto, essas características foram similares nos pacientes do sexo masculino e feminino. Assim, é possível afirmar que os pacientes investigados receberam tempo equivalente de exposição à diálise, eram sedentários, não ingeriam bebidas ancoólicas e apresentavam uma baixa frequência de tabagismo, além de exibir estado nutricional pareado. Em conjunto, esses aspectos reforçam a generalizabilidade dos nossos achados para tota a amostra investigada, independente do sexo e características clínicas, cujas variações pontuais podem exercer limitado impacto sobre os achados gerais frente a homogeneidade na maior parte das características da amostra.

Considerando a melhor caracterização dos pacientes investigados nesse estudo, a estratificação mediante valores de Kt/V revelou diferenças pontuais entre pacientes com dose adequada e inadequada de diálise. Nesse sentido, essa estratificação revelou diferenças bioquímicas restritas à distribuição de leucócitos, uremia pós-diálise e creatinina. Considerando a especificidade do Kt/V em estimar a qualidade da diálise em função da depuração plasmática de solutos de baixo peso molecular (LEVEY & CORESH, 2012), as divergências associadas à ureia e creatinina foram esperadas, reforçando a relevância desse marcador em indicar o clearance dessas toxinas urêmicas (LEVEY & CORESH, 2012). Ao contrário da similaridade nos marcadores clínicos e bioquímicos, a estratificação dos pacientes de acordo com o Kt/V revelou marcantes dintinções nos níveis séricos das citocinas e quimiocinas investigadas. Interessantemente, nós identificamos correlação significativa entre Kt/V e CXCL-10/IP-10. Entretanto, a análise de regressão linear indicou que o Kt/V

apresenta baixa relevância para predizer o comprotamento dessa quimiocina em pacientes expostos à dose adequada ou inadequada de diálise. Esse aspecto reforça a idéia de que outras variáveis clínicas ou bioquímicas não inseridas em nosso modelo analítico podem exercer maior impacto sobre a determinação da inflamação sistêmica em pacientes em hemodiálise, influenciando de forma mais contundente os níveis de CXCL-10/IP-10. Por outro lado, nós identificamos que os níveis de creatinina foram diretamente correlacionados com o acúmulo das moléculas IFN-γ, CCL-2/MCP-1 e CXCL-10/IP-10. No entanto, a creatinina foi inversamente correlacionada aos níveis de IL-10. Nesse contexto, esse metabóito (creatinina) também foi capaz de predizer parcialmente os níveis de todas essas citocinas e quimiocinas quando o Kt/V foi inferior à 1,2. No entanto, essa relevância preditiva foi restrita à IL-10 em condições nas quais os pacientes exibiram dose de diálise adequada.

Mediante monitoramento bioquímico de rotina, verificamos divergênias nos níveis de hemoglobina, ureia pré e pós-HD em nossos pacientes estratificados por sexo. Atualmente, as diretrizem que orientam a avaliação e o tratamento da doença renal crônica não delimitam a influência do sexo sobre alvos clínicos específicos (LOCATELLI *et al*., 2008). No entanto, há evidências de que a variabilidade nos níveis de hemoglobina podem estar relacionada a resposta diferencial à eritropoietina nos diferentes sexos. Nesse sentido, há indícios de que mulheres em hemodiálise podem apresentar uma pior resposta a esse hormônio, repercutindo menores níveis de hemoglobina (IFUDU *et al*., 2001; KHANKIN *et al*., 2010). Assim, essas divergências reforçam a especificidade dos valores de corte para definir anemia de acordo com os níveis de hemoglobina em mulheres e homem, respectivamente estabelecidos em 13,8 e 15,7 g/dL (PALMER *et al*., 2014). De forma análoga, as mulheres apresentaram concentrações de ureia mais baixas do que homens antes e deposis da hemodiálise. O clearance e o acúmulo dessa toxina urêmica representam indicadores metabólicos da biogênese e cinética da ureia que estão diretamente relacionados ao catabólismo proteico

(SILVA *et al*., 2019; HASEGAWA *et al*., 2020). Atualmente, há evidências consistentes de que combinação de indicadores nutricionais e bioquímicos permite obter uma avaliação mais abrangente e robusta do estado catabólico em pacientes com doença renal crônica em estágio terminal, especialmente aqueles em hemodiálise (NKF, 2000; SILVA *et al*., 2019). Como os parâmetros antropométricos e a condição nutricional foram similares em todos os pacientes, independentemente do sexo, acredita-se que a principal distinção entre os sexos pode ser o maior catabolismo protéico em homens. Considerando que esse achado não foi associado à dose de diálise, a influência do sexo sobre os níveis de ureia antes e deposis da hemodiálise requerem investigações adicionais. Assim, parâmetros relevantes como o perfil de ingestão nutricional, os níveis de hidratação e o balanço hormonal merecem ser incorporados em novos estudos, uma vez que exercem influência direta no metabolismo protéico-energético em pacientes renais crônicos (COLMAN *et al*., 2005; PATEL *et al*., 2013).

Além de marcante uremia, pacientes expostos à doses inadequadas de diálise exibiram maior acúmulo de creatinina. Semelhante à ureia, a creatinina também é sistematicamente admitida como um indicador de catabolismo protéico em pacientes sob tratamento hemodialítico. Reconhecidamente, os níveis de creatinina são diretamente influenciados pela ingestão dietética de proteínas, estado nutricional, níveis de hidratação e eficiência da diálise (COLMAN *et al*., 2005; PATEL *et al*., 2013). Na presente investigação, os resultados de creatinina parecem não terem sido influenciados por dietas inadequadas, uma vez que todos os pacientes receberam acompanhamento nutricional pelo menos um ano antes e durante o estudo. Dessa forma, é possível que os resultados de creatinina tenham sido diretamente influenciados pela dose de diálise, mensurada por meio do Kt/V. A creatina é um soluto de retenção urêmica ligada à proteínas, sendo utilizada como um relevante marcador diagnóstico e prognóstico em pacientes dialíticos (COBO *et al*., 2018). Estudos prévios demonstraram que níveis elevados de creatinina circulante estão frequentemente associados ao maior risco de

mortalidade em pacientes em hemodiálise (WANG *et al*., 2017; ILIC *et al*., 2018). Essa relação é complexa, e o acúmulo de ureia e creatinina em pacientes com Kt/V abaixo de 1,2 é consistente com o estresse urêmico, o qual contribui para o desenvolvimento de um microambiente pró-oxidante e pró-inflamatório (COHEN *et al*., 2020; RIOS *et al*., 2017; SYED-AHMED & NARAYANAN, 2019). Curiosamente, o agravamento da uremia pode estar associado ao maior número de leucócitos circulantes em pacientes expostos à doses inadequadas de diálise. No entanto, a fisiopatologia da síndrome inflamatória crônica parece estar especialmente relacionada à retenção de toxinas urêmicas de peso molecular médio, especialmente citocinas e quimiocinas (MIHAI *et al*., 2018; VANHOLDER *et al*., 2018).

Em associação à toxicidade urêmica, os pacientes em hemodiálise apresentaram um marcante estado inflamatório sistêmico, evidenciado pelos elevados níveis de citocinas e quimiocinas em relação aos voluntários saudáveis. Atualmente, o estado pró-inflamatório crônico em pacientes com doença renal crônica terminal é concebido como um síndrome inflamatória persistente e complexa, a qual se desenvolve como resposta ao acúmulo de toxinas urêmicas, condições de diálise e doenças associadas à disfunção renal (RIOS *et al*., 2017; MIHAI *et al*., 2018; SILVA *et al*., 2019). Há evidências de que a síndrome urêmico-inflamatória está associada à patogênese de doenças cardiovasculares em pacientes em hemodiálise, as quais aumentam o risco de mortalidade nesses pacientes em relação à população em geral (ZOU *et al*., 2020; SILVERSTEIN, 2009). Mediante estratificação dos pacientes de acordo com o Kt/V, nós identificamos que doses inadequadas de diálise foram associadas à elevação dos níveis circulantes de citocinas pró-inflamatórias e quimiocinas, bem como redução dos níveis de citocinas anti-inflamatórias (CASTILLO-RODRÍGUEZ *et al*., 2017; RIOS *et al*., 2017). Nesse contexto, doses inadequadas de diálise parecem agravar a inflamação sistêmica crônica em pacientes em hemodiálise, podendo repercutir em maior

risco de mortalidade em pacientes com Kt/V inadequado (CASTILLO-RODRÍGUEZ *et al*., 2017; MILAN MANANI, 2016).

Embora a estratificação pelo Kt/V tenha revelado marcantes diferenças entre pacientes expostos à diferentes doses de dilálise, os valores de Kt/V apresentaram correlação negativa e valor preditivo limitado em relação aos níveis circulantes da quimiocina CXCL-10/IP-10. Além disso, a creatinina foi positivamente correlacionada aos níveis de IFN-γ, CCL-2 / MCP-1 e CXCL-10/IP-10 em pacientes com dose de diálise inadequada. No entanto, creatinina e IL-10 exibiram correlação negativa. Esses achados são relevantes uma vez que indicam que medidas classicamente obtidas na rotina clínica podem ser úteis para estimar a inflamação sistêmica em pacientes sob tratamento hemodialítico. O acompanhamento de mediadores inflamatórios possui utilidade clínica (AKCHURIN & KASKEL, 2015; NOWAK & CHONCHOL, 2018), de modo que ao indicar a condição ou gravidade de comorbidades cardiovasculares, esses mediadores podem auxiliar no monitoramento do risco de mortalidade em pacientes em hemodiálise (COZZOLINO *et al*., 2018; CARRACEDO *et al*., 2020).

Dentre as moléculas investigadas que apresentaram correlação significaticva com o Kt/V e a creatinina; a quimiocina CCL2, também conhecida como MCP-1, consiste de um potente efetor quimioatraente para macrófagos, além de representar um importante indicador de inflamação vascular (DE OLIVEIRA JUNIOR *et al*., 2020). Nesse sentido, elevados níveis de CCL2 foram relevantes indicadores de aterosclerose em pacientes em hemodiálise, cujas propriedades aterogênicas são amplificadas pela presença de elevados níveis de CXCL8 (RUSSO *et al*., 2014). Por sua vez, essa última molécula consiste de um fator quimiotático para neutrófilos intensamente secretado em tecidos inflamados (RUSSO *et al*., 2014), o qual também pode ser utilizado como preditor de mortalidade em pacientes sob tratamento hemodialítico (PANICHI *et al*., 2006). A quimiocina CCL5 também atrai células envolvidas na imunidade inata, especialmente monócitos, células natural killer, basófilos, eosinófilos e

linfócitos T (KRENSKY & AHN, 2007). Existe evidência de que essa quimiocina contribui para o desenvolvimento do fenótipo imunológico Th1, reforçando os mecanismos de ativação da secreção de IFN-γ, o qual é adicionalmente estimulado pelo acúmulo de toxinas urêmicas em pacientes sob tratamento hemodialítico (KRENSKY & AHN, 2007). Como uma típica citocina Th1, IFN-γ é um efetor pró-inflamatório com elevado potencial aterogênico, o qual é mediado pelo papel dessa citocina em estimular a ativação de macrófagos e a produção de óxido nítrico (CARRERO *et al*., 2008; SILVERSTEIN, 2009; MIHAI *et al*. , 2018). Além disso, IFN-γ ativa a secreção de CXCL-10 (HÄGELE *et al*., 2009), quimiocina que pode ser relevante como indicador da gravidade das doenças renais (GAO *et al*., 2020) .

Considerando um efeito oposto àquele provocado pelas citocinas e quimicinas de perfil Th1, as moléculas IL-4 e IL-10 são potentes efetores anti-inflamatórios de perfil Th2 e Treg, respecivamente (CHUNG & LAN, 2011; KANY *et al*., 2019). Essas moléculas estão frequentemente aumentadas em pacientes com doença renal crônica terminal (Chung e Lan, 2011; MAI *et al*., 2020). Entretanto, esse aumento não parece ser suficiente para garantir efeitos anti-inflamatórios consistentes em pacientes em hemodiálise, uma vez que os estímulos pró-inflamatórios são predominantes (GIRNDT *et al*., 2003). Embora apenas uma pequena parcela desses pacientes (cerca de 33%) seja capaz de produzir níveis adequados de IL-4 e IL-10 (GIRNDT *et al*., 2003; COHEN *et al*., 2010), essas moléculas atuam para atenuar a ativação imunológica de efetores Th1 em paciente urêmicos (GIRNDT *et al*., 2003; COHEN *et al*., 2010; AKCHURIN e KASKEL, 2015). Dessa forma, monitorar os níveis de efetores imunológicos Th1 e Th2 pode contribuir para avaliar o balanço entre o estado pró-inflamatório e anti-inflamatório em pacientes em hemodiálise (GU *et al*., 2020; AKCHURIN & KASKEL, 2015). Infelizmente, a dosagem desses efetores é frequentemente inviável na rotina clínica. Assim, modelos preditivos baseados na utilização de marcadores frequentemente monitorados nos centros de hemodiálise podem oferecer alternativas

relevantes para estimar a relação entre sobrecarga urêmica e o estado inflamatório nos pacientes em hemodiálise. A partir dessas estimativas, o manejo do paciente em hemodiálise pose ser reestruturado, em busca de melhores resultados terapêuticos com vistas à atenuação da síndrome urêmico-inflamatória para aumentar as taxas de sobrevivência do paciente com doença renal terminal sob tratamento hemodialítico.

6 CONCLUSÃO

A partir da presente investigação clínica transversal, foi possível identificar que pacientes em hemodilálise apresentam elevados níveis circulantes de citocinas e quimiocinas comparados à voluntários saudáveis, caracterizando um marcante estado pró-inflamatório sistêmico nesses pacientes. Por meio do Kt/V, foi possível estratificar os pacientes com dose de diálise adequada e inadequada. Essa estartificação indicou que pacientes com dose inadequada de diálise apresentam dose inadequada de diálise apresentam aumento do número de leucócitos circulantes, dos npiveis de ureia e creatinina pós-diálise. Além disso, esses pacientes também apresentaram níveis aumentados de citocinas e quimiocinas próinflamatórias, e níveis reduzidos de moléculas anti-inflamatórias, indicando que a dose de diálise pode influenciar no acúmulo desses efetores imunológicos. Além disso, os resultados o Kt/V foi inversamente correlacionado com os níveis circulantes de CXCL-10/IP-10, enquanto os níveis de creatinina foram diretamente correlacionados com os níveis de IFN-γ, CCL-2 / MCP-1 e CXCL-10 / IP-10, e inversamente correlacionados com os níveis circulantes de IL-10. Apesar dessas relações, o Kt/V e creatinina exibiram limitada relevância preditiva para as citocinas e quimiocinas investigadas. Assim, ainda se faz necessário identificar preditores mais sensíveis e específicos de sobrecarga urêmico-inflamatória em pacientes em hemodiálise.

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8 ANEXO I – Artigo publicado

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Cytokines and chemokines systemic levels are related to dialysis adequacy and creatinine clearance in patients with end-stage renal disease undergoing hemodialysis

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Uremic toxins

ABSTRACT

Although the clearance of low-molecular weight toxins is modulated by dialysis dose, the relationship between dialysis adequacy and middle systemic inflammatory mediators is often overlooked. Thus, the relationship between dialysis adequacy, pro- and anti-inflammatory cytokines and chemokines in hemodialysis (HD) patients was investigated. Forty-eight HD patients (19 women and 25 men) were investigated. Age, body mass index, time in HD, nutritional status, Kt/V and blood biochemical parameters was similar in patients of both sexes ($P > 0.05$). Thus, patients were stratified by dialysis adequacy measured by Kt/V method (adequate Kt/V ≥ 1.2). Post-HD urea, creatinine, cytokines (IFN-γ, IL-4 and IL-10) and chemokines (CCL-2, CCL-5, CXCL-8 and CXCL-10) were higher in patients with Kt/V < 1.2 ($P < 0.05$). Kt/V exhibited significant correlation with CXCL-10/IP-10 serum levels. Positive correlation between creatinine with IFN-γ, CCL-2/MCP-1, and CXCL-10/IP-10, and negative correlation with IL-10 was identified in patients with Kt/V < 1.2 ($P < 0.05$). In patients with Kt/V ≥ 1.2, only IL-10 was positively and CXCL-10/IP-10 negatively correlated with creatinine levels ($P < 0.05$). Kt/V and creatinine levels exhibited variable predictive value (Kt/V = 27% to 37%, creatinine = 29% to 47%) to explain cytokines and chemokines circulating levels in patients with adequate and inadequate dialysis dose. Taken together, our findings provide evidence that in addition to modulating uremic toxins levels, such as urea and creatinine, dialysis dose is associated with circulating levels of inflammatory mediators. Thus, low Kt/V results and creatinine accumulation are potential indicators of the systemic inflammatory stress determined by up-regulation of proinflammatory cytokines and chemokines, and downregulation of anti-inflammatory cytokines.

1. Introduction

Chronic kidney disease (CKD) is an impacting public health problem worldwide, causing high social and economic costs to the health system [1]. Epidemiological evidences indicated a global prevalence of 753 million people with CKD (i.e. 417 million females and 336 million males) in 2016 [2]. This disease is associated with high morbidity and mortality, especially in poor countries due to the limited access to specialized and more expensive treatments, such as renal replacement therapies [3]. Thus, renal failure has been responsible by over 1 million deaths/year in developing countries [4,5]. CKD etiology is complex and often multifactorial. However, systemic arterial hypertension, diabetes mellitus, renal infections, and chronic immunomediated diseases are the main comorbidities associated with the high incidence, prevalence and severity of CKD worldwide [6,7].

In more severe cases and in the absence of adequate treatment, the patient with CKD progresses to end-stage renal disease (ESRD), requiring renal replacement therapy (i.e., hemodialysis [HD] or

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