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ANDERSON DE CASTRO RIBEIRO

TEMPORAL DYNAMICS IN CARDIOMETABOLIC RISK FACTORS, BIOCHEMICAL AND IMMUNOLOGICAL INDICATORS IN PATIENTS UNDERGOING RENAL REPLACEMENT THERAPY

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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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“Se eu vi mais longe, foi por estar sobre ombro de gigantes.”

(Isaac Newton, 1675).

ABSTRACT

Hemodialysis patients (HP) are exposed to malnutrition, cardiometabolic and pro-inflammatory risk factors. However, limited knowledge of variability in these risk factors remains a central limitation for adequate clinical management of HP. From a longitudinal study, we investigated the relationship between time-dependent variability in cardiometabolic risk factors and biochemical markers with cytokines and adipokines circulating levels in HP. Thirty-eight HP (women = 15, men = 23) aged 54.13 ± 16.78 years old underwent three independent anthropometric, nutritional, biochemical and immunological assessments (1, 6 and 12 months). Patient's characteristics (body mass, comorbidities, history of kidney disease and time on hemodialysis) were similar after sex stratification. From grouped data, 31.6 to 100.0% HP exhibited multiple malnutrition and cardiometabolic risk factors in all time-points evaluated. All anthropometric and nutritional results and most biochemical markers were similar in 1, 6 and 12 months follow-up, indicating a marked time-dependent stability. Urea, creatinine, total proteins, albumin, adipokines (adiponectin, leptin and resistin) and cytokines (TNF, IL-6 and IL-10) levels were highly variable in 12 months follow-up. Direct correlations between leptin and fat mass, TNF and IL-6 with creatinine and pre-dialysis urea were observed in all time-points (1, 6 and 12 months). Creatinine and pre-dialysis urea were negatively correlated with IL-10 for the entire follow-up. Fat mass, creatinine and pre-dialysis urea were predictive markers of leptin, TNF, IL-6 and IL-10 variability. Our findings indicated that biochemical, nutritional and cardiovascular risk factors exhibit low time-dependent variability in HP under clinical and nutritional monitoring. However, adipokines and cytokines are highly variables, which can potentially be influenced by body adiposity, creatinine and urea clearance. Thus, these parameters can contribute to predict the inflammatory status in HP.

Keywords – Malnutrition; hemodialysis; inflammation; kidney disease.

RESUMO

Pacientes em hemodiálise (PH) estão expostos a desnutrição, fatores de risco cardiometabólicos e pró-inflamatórios. No entanto, o conhecimento limitado da variabilidade desses fatores de risco continua sendo uma limitação para o manejo clínico desses pacientes. A partir de um estudo longitudinal, investigamos a relação entre a variabilidade de fatores de risco cardiometabólicos e de marcadores bioquímicos com os níveis de citocinas e adipocinas em PH. Trinta e oito pacientes (mulheres = 15, homens = 23) com idade de 54,13 ± 16,78 anos foram submetidos a três avaliações antropométricas, nutricionais, bioquímicas e imunológicas independentes (1, 6 e 12 meses). As características dos pacientes (massa corporal, comorbidades, história de doença renal e tempo em hemodiálise) foram semelhantes após a estratificação por sexo. 31,6% à 100,0% dos PH exibiram múltiplos fatores de risco cardiometabólicos e de desnutrição em todos os momentos avaliados. Todos os resultados antropométricos e nutricionais, bem como a maioria dos marcadores bioquímicos foram semelhantes em 1, 6 e 12 meses de acompanhamento ($P>0.05$), indicando uma marcante estabilidade tempo-dependente. Entretanto, os níveis de uréia, creatinina, proteínas totais, albumina, adipocinas (adiponectina, leptina e resistina) e citocinas (TNF, IL-6 e IL-10) foram altamente variáveis no seguimento de 12 meses ($P<0.05$). Correlações diretas entre leptina e massa gorda, e entre TNF e IL-6 com creatinina e uréia pré-diálise foram observadas em todos os momentos avaliados ($P<0.05$). Creatinina e uréia pré-diálise foram negativamente correlacionadas com IL-10 em todo o seguimento ($P<0.05$). Massa gorda, creatinina e uréia pré-diálise foram marcadores preditivos da variabilidade da leptina, TNF, IL-6 e IL-10 ($P<0.05$). Os achados indicaram que fatores de risco bioquímicos, nutricionais e cardiomtabólicos exibem baixa variabilidade tempo-dependente em PH. No entanto, adipocinas e citocinas são altamente variáveis, sendo potencialmente influenciadas pela adiposidade corporal, depuração creatinina e de uréia. Assim, esses parâmetros podem contribuir para predizer o estado inflamatório em PH.

Palavras-chave – Desnutrição; hemodiálise; inflamação; doença renal.

SUMMARY

1 INTRODUCTION

Chronic kidney disease (CKD) is a serious and growing public health problem that affects 10 to 12% of the world's population (GBD, 2020). In addition to genetic risk factors, the presence of comorbidities such as diabetes mellitus, systemic arterial hypertension, infectious and autoimmune diseases are directly linked to the higher incidence and prevalence of CKD (Chen et al., 2019). In more severe cases, deterioration in renal structure and function determines progression to end-stage renal disease (ESRD), which invariably requires renal replacement therapy, especially hemodialysis or kidney transplantation (Cobo et al., 2018). In addition to the high socioeconomic burden associated with the treatment, patients with ESRD require intensive interdisciplinary treatment, which often involves psychological, nutritional and medical care (Chen et al., 2019; Johns et al., 2015).

Epidemiological studies indicate that ESRD patients have a higher frequency of hospitalization, nutritional and metabolic disorders, as well as mortality rates that are 100 to 200 times higher compared to the general population (Castillo-Rodríguez et al., 2017). Due to renal failure and the accumulation of toxic metabolites, patients with ESRD manifest a uremic phenotype often linked to the development of a systemic pro-inflammatory status, which is consistently associated with the high prevalence of nutritional and cardiovascular disorders in individuals undergoing hemodialysis (Cobo et al., 2018; Cohen, 2020). ESRD prognosis is poor in the absence of adequate treatment, and most patients eventually die from cardiovascular complications, infections or progressive uremia (e.g., metabolic acidosis and severe malnutrition) (Chen et al., 2019; Cohen, 2020).

Several evidences demonstrates that no single measure provides a complete and unambiguous assessment of ERSD patients undergoing hemodialysis (Ashby et al., 2019; Bigogno et al., 2014). Thus, the integration of a broad panel of clinical, biochemical and nutritional indicators is essential to improve the monitoring of treatment efficacy and to delimit a more accurate prognosis for these patients (Balbino et al., 2019). Indicators such as hemodialysis dose, serum creatinine and albumin levels, nutrient intake profile, global nutritional status, interdialytic weight gain, protein energy wasting, and lean/fat mass ratio have been associated with the risk of morbidity and mortality in ERSD (Ashby et al., 2019; Balbino et al., 2019; Noori et al., 2010; Okuno, 2021). In addition to presenting a strong reciprocal correlation (Bigogno et al., 2014; Okuno, 2021), these parameters are associated with the inflammatory state in hemodialysis patients, which has received increasing attention

as it is considered a major component of the uremic phenotype linked to cardiometabolic risk in this population (Cobo et al. al., 2018; Cohen, 2020).

The etiology of chronic systemic inflammatory syndrome in hemodialysis patients is complex, multifactorial and still poorly understood. Dialysis-related factors (e.g., vascular catheter and dialysate contamination, exposure to endotoxins, dialysate reflux through the dialysis membrane, and low biocompatibility of this membrane) are recognized as important sources of persistent low-grade pro-inflammatory stimuli (Cobo et al., 2018; Pertosa et al., 2000). However, the systemic inflammatory profile is particularly influenced by the accumulation of uremic toxins, since dialysis has limited efficiency in removing excess pro-inflammatory molecules with molecular mass >10 kD, such as several cytokines and adipokines (Cobo et al., 2018; Wolley and Hutchison, 2018). Accordingly, direct immunomodulators such as IL-6, TNF, adiponectin, and resistin are recognized as uremic toxins, which seem to orchestrate the cardiometabolic instability of hemodialysis patients (Cobo et al., 2018).

Previous studies linked high levels of cytokines such as IL-1, IL-6, and TNF with negative clinical outcomes (e.g., hypotension, fever, bone disease, anemia, anorexia, malnutrition, atherogenesis, vascular calcification, and attenuation of the immune response against infectious agents), as well as increased cardiovascular and death risk in hemodialysis patients (Bologa et al., 1998; Pertosa et al., 2000). Furthermore, increased leptin, resistin and adiponectin circulating levels have been reported in ESRD patients (Lim et al., 2015), which are variably associated with an increase (Marouga et al., 2016) or reduction (Scholze et al., 2007) in the risk of morbidity and mortality in ESRD patients. Thus, the impact of such adipokines remains controversial in this population, especially considering that they can activate (Martinez Cantarin et al., 2014) or inhibit (Miyamoto and Sharma, 2013) pro-oxidant and pro-inflammatory molecular pathways directly involved in ESRD pathogenesis.

Cytokines and adipokines circulating levels are often associated with the presence of comorbidities, nutritional profile and body composition in general population (Arroyo-Jousse et al., 2020). However, limited knowledge about the variability of these inflammatory effectors in ESRD remains a central limitation for the proper assessment, delimitation of treatment strategies, and clinical follow-up of hemodialysis patients. Currently, it is recognizable that malnutrition, metabolic imbalance, and systemic inflammatory response syndrome coexist and have a bidirectional cause-effect relationship in uremic patients (Peev and Nayer, 2014). Thus, we conducted a prospective longitudinal study to elucidate the

relationship between cardiometabolic and biochemical markers with cytokine and adipokines levels in ESRD patients undergoing hemodialysis.

2 GENERAL OBJECTIVE

Investigate the relationship between time-dependent variability in nutritional/cardiometabolic risk factors, biochemical markers, cytokine and adipokines circulating levels in ESRD patients undergoing hemodialysis.

2.1 SPECIFIC OBJECTIVES

- To compare clinical characteristics of ESRD patients undergoing hemodialysis according to sex;
- To investigate the variability in nutritional, antropometric and biochemical markers in ESRD patients undergoing hemodialysis in a 12-months follow-up;
- To investigate the variability in cytokine and adipokines circulating levels in ESRD patients undergoing hemodialysis in a 12-months follow-up;
- To evaluate potential correlations between nutritional, antropometric and biochemical markers with cytokine and adipokines circulating levels in ESRD patients undergoing hemodialysis in a 12-months follow-up.

3 PATIENTS AND METHODS

3.1 STUDY DESIGN, SAMPLE SIZE AND DIALYSIS CONDITIONS

This is a longitudinal prospective study (12-months follow-up) with all adult ESRD patients of both sexes undergoing hemodialysis (HD) in the Renal Replacement Therapy Center of Alzira Velano University Hospital. Patients of both sexes that agreed to participate in the study and signed the free consent term were included. Exclusion criteria were: i) patients who refuse to participate in the study, ii) presence of cognitive deficit (evaluated by the Mini Mental State Examination) that difficult the application of the questionnaires (Brucki et al., 2003), iii) patients submitted to renal transplantation during the last 6 months, iv) neoplastic disease, vi) change in dialysis modality during the last 3 months, vii) newly implanted catheters, viii) hemodynamic instability, and ix) patients with physical incapacity to stay in standing position for anthropometric evaluation (Silva et al., 2019). It is worth mentioning that no patient were excluded based on the above mentioned criteria. Thus, all patients (n= 49) undergoing (HD) from a Renal Replacement Therapy Center were included in this study. All patients received 3-4h HD sessions three times a week. Blood flow was established at 300-450 mL/min with a dialysate stream at 500 mL/min constant rate. Dialysis was based on high flux polysulfone membranes with bicarbonate-buffered dialysate and low flow polysulfone membranes.

3.2 STUDY PROTOCOL AND ETHICAL ISSUES

The same nephrologist (R.E.S.) and nutritionist (P.B.I.J.) who were responsible for clinical follow-up of the patients performed all data collection. Data were collected at three different times (months 1, 6 and 12) over a 12-month follow-up. All records were obtained during the first two of the three weekly HD sessions performed by each patient. In the first session, the general characteristics of the patients (i.e., age, sex, comorbidities, time on hemodialysis, smoking, alcohol intake, and Kt/V) were collected from the medical record (weekly updated) and confirmed with all patients, when appropriated. The nutritional assessment was also performed in the first week session to ensure a more detailed food recall, which considered food intake in a typical week, including weekends. To avoid the influence of the HD procedure on blood parameters, blood samples were collected using Gel SST II Advance Vacutainer® tubes (Becton Dickinson, San Jose, CA, USA) immediately before the

second session (48h after the first HD session). Immediately after this session, the anthropometric parameters were recorded to avoid the influence of water retention on body composition. All measurements were performed in triplicate and the mean values were calculated. 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).

3.3 NUTRITIONAL STATUS AND MALNUTRITION ASSESSMENT

Malnutrition was investigated in all subjects from a multilevel clinical and nutritional screening, including: (i) modified global subjective assessment - mGSA for HD patients (Ruperto et al., 2016), and (ii) anthropometric measures obtained after HD session (dry weight [kg], height [cm], waist, hip and arm circumferences [cm], and skinfold thickness (triceps, biceps, subscapular and suprailiac) according standardized protocols (WHO, 1995; NKF, 2000; Duarte 2007; Silva et al., 2019). Waist circumference (WC) was measured at the expiratory phase from the abdominal point with the largest circumference. Waist circumference was classified according to the cut-off points described by the World Health Organization (WHO, 1995). Hip circumference (HC) was evaluated without tissue compression at the most protuberant point in the horizontal alignment between hips and buttocks (Duarte 2007). From the waist/hip ratio (WHR), the risk of cardiovascular disease was estimated considering recommended cut-off points (WHO, 1995). Waist/height ratio (WHtR) measured by the ratio between the WC (cm) and the height (cm) (Vidigal et al., 2015); conicity index (CI) was calculated using the equation CI= WC (m) / (0.109 x [Body weight (kg) / Height (m)]$^{1/2}$) (Valdez et al., 1991), and body adiposity index (BAI) as calculated as BAI= (HC[cm] / (height [m]* height [m]$^{1/2}$) -18 (Bergman et al., 2011).

Skinfold thickness was measured in triplicate by means of the skinfold caliper with precision from 0 to 60 mm, scale of 1 mm and constant pressure of 10g / mm^2 (Lange, Ann Arbor, MI, USA) (NRC, 1988). Arm circumference was analyzed with the upper limbs parallel to trunk, and the midpoint between the scapula acromion and the olecranon was used as reference for the measures. The reference values of U.S. Hanes were used to evaluate the adequacy of our arm circumference results (Frisancho, 1981). Arm fat area (AFA) (Frisancho, 1981), and adjusted-arm muscle area (AAMA) (Heymsfield et al., 1982; Barbosa et al., 2014) were calculated from the results of triceps skinfold thickness and arm circumference. Body fat

percentage was estimated considering the sum of four skinfolds thickness (Σ4ST= suprailiac + biceps + triceps + subscapular) (Durnin et al., 1974; Pereira et al., 2013).

3.4 CUT-OF POINTS OF MALNUTRITION

Malnutrition was classified according objective criteria for each method: (i) mGSA: 9–23 points = mild malnutrition/nutritional risk, 24-31 points = moderate malnutrition, 32-39 points = severe malnutrition, ≥ 40 points = very serious malnutrition (Vos et al., 2015). (ii) BMI: <18.5 kg/m^2 (underweight), 18.6-24.9 kg/m^2 (eutrophic), >25 kg/m^2 (overweight) for adults (WHO, 1995); and <22 kg/m^2 (underweight), 22-27 kg/m^2 (eutrophic) and >27 kg/m^2 (overweight) for the elderly (Vos et al., 2015). (iii) Arm circumference (AC): Arm muscle circumference and triceps skinfold thickness: 50th percentile as reference according age and sex (Chumlea et al., 1998). Classification: <70% = severe malnutrition, 70-80% = moderate malnutrition, 80-90% = mild malnutrition (Riella and Martins, 2001). (iv) Waist circumference (WC): ≥80 cm for women or ≥94 for men indicates high risk of metabolic complications, and ≥88 cm for women or ≥102 indicates very high risk. (v) WHR: >1.0 for men and >0.85 for women (WHO, 1995). (vi) Adjusted-arm muscle area (AAMA): according age and sex. Classification: <5th percentile = severe malnutrition and >5th and <15th = mild/moderate malnutrition. (vii) Arm fat area (AFA): <25 percentile (Balbino et al., 2017), admitting the 25th percentile for age and sex (WHO, 1995). (xii) Skinfold-based percent body fat (PBF): Fat shortage for woman/men ranging from 18-39 years (<21% / 8%), 40–59 years (<23% / 11%), 60–99years (<34% / 13%); and excess fat for woman/men ranging from 18-39 years (<33% / 20%), 40–59 years (<34% / 22%), 60–99years (<36% / 25%) for men (Balbino et al., 2019).

3.5 DIETARY AND NUTRIENT INTAKE ASSESSMENT

A standardized 24-hour food record was used to collect information about dietary intake (Barufaldi et al., 2016). We used the standardized technique described by the authors, in which the respondent was stimulated to remember the food they consumed the day before. During the evaluation, all patients were instructed to record daily drink and food intake. Dietary intake data were analyzed by using DietBox software to estimate energy and nutrient consumption. This software uses the Brazilian Table databases Food Composition 2011 - TACO (NEPA, 2011), Composition Table of the Brazilian Institute of Geography and

Statistics (IBGE - Brazil) and the Food Composition Table Tucunduva (Philippi, 2016), with more than 5 thousand registered foods. Detailed instructions were given to all patients to record all dietary habits including additions and amount (i.e. sugar, salt, and oils), supplementations (i.e. vitamin and mineral), cooking method, type and brand names of industrial foods. Conventional measures such as cups, spoons and bowls, as well as portion sizes (small, medium and large) were defined and recorded to help the nutritional analysis (Barbosa et al., 2014). Daily food consumption was estimated as frequency × portion × size for each consumed food item. Nutrient intake was evaluated according to two Brazilian nutritional composition tables (NEPA, 2011; Philippi, 2012) or to an international reference table (USDA, 2017), when the nutritional information was unavailable in Brazilian tables (Cocate et al., 2015).

3.6 BLOOD BIOCHEMICAL PARAMETERS AND DIALYSIS DOSE

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. Urea, creatinine, iron, calcium, potassium, phosphorus, total protein, albumin, alkaline phosphatase, glucose, parathyroid hormone, total iron-binding capacity, cholesterol and fractions were analyzed by spectrophotometry using commercial diagnostic colorimetric kits (Labtest, Itabira, MG, Brazil). Transferrin saturation index (TSI, %) was calculated as follows: TSI = (serum iron level × 100%) / total iron-binding capacity (Beilby et al., 1992). Hemoglobin, hematocrit, leucocytes, and platelets were quantified in total blood samples using a hematological analyzer and high-grade human reagents (Sysmex, XE-2100, Sao Paulo, SP, Brazil). The dialysis dose was calculated by the Kt/V equation: 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. Urea reduction ratio (URR) after dialysis was estimated according the relation URR = ([U*pre* – U*post*]/U*pre*)*100% (Silva et al., 2019).

3.7 CYTOKINES AND ADIPOKINES IMMUNOASSAY

The cytokines interleukin-6 (IL-6), interleukin-10 (IL-10) and tumor necrosis factor (TNF) were quantified in serum samples by flow cytometry bead array (CBA). Commercial kits for human cytokines 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 at 20 to 5000 pg/mL. The lower limit of CBA-based cytokines detection was 2.6 to 18.9 pg/mL according to the analyte considered. The adipokines resistin, leptin, and adiponectin were measured in serum samples by spectrophotometry from 96-wells commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits, following the manufacturer's instructions (ThermoFisher Scientific, Waltham, Massachusetts, USA). Each kit presented different detection range for resistin (31-2,000 pg/mL), leptin (15.6-1000 pg/mL), and adiponectin (500-32,000 pg/mL).

3.8. STATISTICAL ANALYSIS

Data were expressed as frequencies, mean and standard deviation or median, depending on the variable's distribution, which was analyzed by D'Agostino-Pearson test. Clinical characteristics according sex were compared by Student's t test or Mann-Whitney U test for continuous variables, and Pearson's chi-squared test or Fisher's exact test for categorical variables. One-way analysis of variance (one-way ANOVA) for parametric data and Kruskal-Wallis one-way ANOVA on Ranks for nonparametric data followed by Student-Newman-Keuls *post-hoc* test were applied to compare anthropometric parameters, body composition, nutrient intake, biochemical and immunological data, which were collected at 3 different times (1, 6 and 12 months). Macronutrients and micronutrients intake was adjusted by body mass. The correlation of data showing significant differences in at least two independent evaluations was analyzed by Pearson's test and linear regression. Statistical results with $P \leq 0.05$ were considered statistically significant in all tests.

4 RESULTS

Of the 49 patients recruited, 38 completed the 12-month segment and were included in all analyzes. Thus, 11 patients were excluded due to kidney transplantation (n = 6), transfer to another health center (n = 3), and change in dialysis modality (n = 2). Stratified by sex, both groups of patients exhibited similar distribution of body mass, comorbidities, frequency of smoking and drinking, history of kidney disease and time on hemodialysis (Table 1). Based on this similarity, the variability in anthropometric, biochemical, nutritional and immunological data was assessed from the data grouped for the entire sample analyzed.

Table 1 - General characteristics of patients with end-stage renal disease undergoing hemodialysis.

Variables	Total (n= 38)	Women (n= 15)	Men (n= 23)	*P value*
Age (years), mean ± S.D.				
	54.13 ± 16.78	56.13 ± 16.71	52.83 ± 17.07	*0.560*[(a)]
Body mass (kg), mean ± S.D.				
	67.17 ± 15.37	63.57 ± 15.20	69.53 ± 15.35	*0.248*[(a)]
Body mass index, mean ± S.D.				
	25.33 ± 5.50	26.27 ± 6.47	24.72 ± 4.82	*0.403*[(a)]
Comorbidities, n (%)				
SAH	23 (60.53)	8 (53.33)	15 (65.22)	*0.499* [(b)]
DM + SAH	7 (18.42)	2 (13.33)	5 (21.74)	
SAH + CHF	5 (13.16)	3 (20.00)	2 (8.70)	
Others	3 (7.90)	2 (13.33)	1 (4.35)	
Smoking, n (%)				
Yes	8 (21.05)	1 (6.67)	7 (30.45)	*0.114* [(b)]
No	30 (78.95)	14 (93.33)	16 (69.57)	
Alcohol intake, n (%)				
Yes	0 (0)	0 (0)	0 (0)	*1.00*[(b)]
No	38 (100)	15 (100)	23 (100)	
Family history of kidney disease, n (%)				
Yes	8 (21.05)	2 (13.33)	6 (26.09)	*0.439*[(b)]
No	30 (78.95)	13 (86.67)	17 (73.91)	
Time in hemodialysis (years), mean ± S.D.				
	4.35 ± 2.95	4.17 ± 2.93	4.64 ± 3.10	*0.676*[(a)]

Source: From author (2021).

Subtitles: DM, diabetes mellitus; SAH, systemic arterial hypertension; Other comorbidities = systemic lupus erythematosus (n= 1), hepatitis C (n= 2). 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.

As indicated in Table 2, the characterization of the patients' nutritional status was initially based on a wide anthropometric assessment. All the measured anthropometric parameters showed similar results (P>0.05) in the three independent assessments (month 1, 6 and 12). The distribution of fat and lean mass also showed evident time-dependent stability between the three assessments (P>0.05).

Table 2 - Anthropometry-based nutritional indicators in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Variables	Evaluation 1 n (%)	Evaluation 2 n (%)	Evaluation 3 n (%)	*P value*
Body mass, kg	67.17 ± 15.37	67.88 ± 15.44	68.18 ± 16.00	*0.971* [(a)]
Height, cm	1.64 ± 0.10	1.64 ± 0.10	1.64 ± 0.09	*0.069* [(b)]
Body mass index, kg/m^2	25.33 ± 5.50	25.51 ± 5.70	25.52 ± 5.72	*0.949* [(a)]
Waist circumference, cm	91.13 ± 17.17	90.47 ± 17.57	88.87 ± 17.70	*0.747* [(a)]
Hip circumference, cm	97.05 ± 11.84	95.95 ± 12.04	97.46 ± 11.57	*0.747* [(a)]
Arm circumference, cm	29.34 ± 4.34	29.14 ± 4.71	28.46 ± 4.75	*0.952* [(a)]
Waist/Height ratio	0.56 ± 0.11	0.56 ± 0.11	0.55 ± 0.11	*0.833* [(a)]
Waist/Hip ratio	0.94 ± 0.13	0.94 ± 0.11	0.90 ± 0.14	*0.233* [(b)]
Biceps ST (mm)	9.74 ± 6.00	10.13 ± 7.65	10.55 ± 6.98	*0.856* [(a)]
Suprailiac ST (mm)	12.71 ± 6.20	14.18 ± 8.35	14.22 ± 7.71	*0.604* [(a)]
Triceps ST (mm)	15.29 ± 7.49	15.45 ± 8.12	16.58 ± 8.21	*0.701* [(a)]
Subscapular ST (mm)	15.47 ± 7.89	15.82 ± 8.13	16.34 ± 8.18	*0.877* [(a)]
Fat mass Σ4ST (%)	25.66 ± 8.87	26.30 ± 8.91	26.78 ± 8.23	*0.856* [(a)]
Lean mass Σ4ST (%)	74.34 ± 8.95	73.62 ± 9.40	73.01 ± 8.77	*0.849* [(a)]
Fat mass Σ4ST (kg)	15.53 ± 7.84	18.38 ± 8.06	18.87 ± 8.09	*0.760* [(b)]
Lean mass Σ4ST (kg)	49.69 ± 11.30	49.68 ± 11.51	49.76 ± 10.90	*0.999* [(b)]
Body adiposity index	28.68 ± 7.54	28.69 ± 7.58	28.91 ± 7.50	*0.927* [(a)]
Conicity index	1.31 ± 0.19	1.29 ± 0.19	1.27 ± 0.18	*0.362* [(a)]
AAMA (cm^2)	48.51 ± 16.18	48.52 ± 14.40	45.36 ± 14.82	*0.579* [(b)]
Arm fat area (cm^2)	20.13 ± 11.90	21.30 ± 13.41	22.42 ± 16.06	*0.644* [(a)]

Source: from author (2021).

Subtitles: Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). BMI: body mass index; WC: waist circumference; HC: Hip circumference; WHR: waist/hip ratio; WHtR: waist/height ratio; AC: arm circumference; AAMA: adjusted-arm muscle area; AFA: fat arm area; mGSA: modified global subjective assessment; BAI: body adiposity index; CI: conicity index. P values represent the result of [(a)] Kruskal-Wallis one-way ANOVA on Ranks followed by Student-Newman-Keuls *post-hoc* test, and [(b)] One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test.

From well-defined cut-off points and the modified global subjective assessment (mGSA), cardiometabolic and malnutrition risk in patients undergoing hemodialysis were reported in

Table 3. All indicators used demonstrate some malnutrition and cardiovascular risk in the sample investigated. Risk rates were mainly demonstrated from the percentage of body fat, waist circumference, and mGSA. Risk of malnutrition in at least 94.74% of patients was indicated based on the latter indicator (mGSA). Regardless of the indicator used, the cardiometabolic and malnutrition risk rates were similar when comparing the three assessments performed ($P>0.05$).

Table 3 - Cardiometabolic and malnutrition risk in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Variables	Evaluation 1 n (%)	Evaluation 2 n (%)	Evaluation 3 n (%)	*P value*
BMI				
Underweight	7 (18.42)	5 (13.16)	4 (10.53)	
Adequate weight	16 (42.11)	18 (47.37)	20 (52.63)	*0.850*
Overweight	15 (39.47)	15 (39.47)	14 (36.84)	
WC				
Adequate nutrition	17 (44.74)	16 (42.11)	17 (44.74)	
High risk	5 (13.16)	8 (21.05)	7 (18.42)	*0.921*
Very high risk	16 (42.11)	14 (36.84)	14 (36.84)	
WHR				
No risk	20 (52.63)	20 (52.63)	22 (57.90)	*0.868*
Risk	18 (47.37)	18 (47.37)	16 (42.11)	
AC				
Adequate nutrition	20 (52.63)	19 (50.00)	19 (50.00)	
Mild malnutrition	6 (15.79)	5 (13.16)	6 (15.79)	
Moderate malnutrition	4 (10.53)	6 (15.79)	6 (15.79)	*0.985*
Severe malnutrition	3 (7.89)	4 (10.53)	3 (7.89)	
Overweight/Obesity	5 (13.16)	4 (10.53)	4 (10.53)	
AAMA				
Adequate nutrition	26 (68.42)	24 (63.15)	22 (57.99)	
Mild/Moderate malnutrition	2 (5.26)	4 (10.53)	3 (7.89)	*0.818*
Severe malnutrition	10 (26.32)	10 (26.32)	13 (34.21)	
AFA				
Adequate nutrition	24 (63.15)	24 (63.15)	20 (52.63)	
Mild malnutrition	4 (10.53)	5 (13.16)	4 (10.53)	*0.754*
Moderate malnutrition	10 (26.32)	9 (23.68)	14 (36.84)	
%BF				
Fat shortage	4 (10.53)	6 (15.79)	4 (10.53)	
Normal fat	18 (47.37)	16 (42.11)	14 (36.84)	*0.793*
Excess fat	16 (42.11)	16 (42.11)	20 (52.63)	
mGSA				
Appropriate	2 (5.26)	0 (0.0)	0 (0.0)	*0.131*
Risk/mild malnutrition	36 (94.74)	38 (100)	38 (100)	

Source: from author (2021).

Subtitles: Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). BMI: body mass index; WC: waist circumference; WHR: waist-hip ratio; AC: arm circumference; AAMA: adjusted-arm muscle area; AFA: arm fat area; %BF: skinfold-based percent body fat; mGSA: modified global subjective assessment. P values represent the result of Fisher's exact test for categorical variables.

The nutritional intake profile was assessed based on a 24-hour recall, and is shown in Table 4. All data were normalized according body mass. In general, the intake of macro and micronutrients was similar in all three moments evaluated (1, 6 and 12 months).

Table 4 - Adjusted nutrients intake† in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Variables[#]	Evaluation 1	Evaluation 2	Evaluation 3	*P value*
Dietary intake (g/kg)	17.76 ± 8.94	17.45 ± 10.07	17.90 ± 14.72	*0.612* [(a)]
Energy intake (Kcal/kg)	23.31 ± 24.32	20.24 ± 11.93	20.47 ± 10.72	*0.863* [(a)]
Protein (g/kg)	0.97 ± 0.56	0.90 ± 0.54	0.91 ± 0.45	*0.827* [(b)]
Carbohydrate (g/kg)	3.31 ± 5.64	2.55 ± 1.66	2.53 ± 1.93	*0.951* [(a)]
Lipid (g/kg)	0.70 ± 0.47	0.72 ± 0.49	0.68 ± 0.38	*0.996* [(a)]
Fibers (g/kg)	0.011 ± 0.008	0.012 ± 0.008	0.011 ± 0.007	*0.986* [(a)]
Saturated fat (g/kg)	0.21 ± 0.16	0.23 ± 0.17	0.21 ± 0.15	*0.940* [(a)]
Monounsaturated fat (g/kg)	0.22 ± 0.17	0.21 ± 0.18	0.19 ± 0.13	*0.749* [(a)]
Polyunsaturated fat (g/kg)	0.14 ± 0.10	0.13 ± 0.12	0.13 ± 0.09	*0.621* [(a)]
Cholesterol (mg/kg)	2.74 ± 2.15	2.74 ± 2.57	2.40 ± 1.73	*0.921* [(a)]
Sodium (mg/kg)	24.16 ± 13.39	22.02 ± 13.74	24.66 ± 13.59	*0.237* [(a)]
Iron (mg/kg)	0.14 ± 0.07	0.13 ± 0.07	0.15 ± 0.09	*0.350* [(a)]
Potassium (mg/kg)	25.01 ± 12.15	25.95 ± 25.71	22.63 ± 10.05	*0.504* [(a)]
Magnesium (mg/kg)	2.57 ± 1.38	3.29 ± 5.73	2.31 ± 1.01	*0.606* [(a)]
Manganese (mg/kg)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	*0.949* [(a)]
Phosphorus (mg/kg)	11.46 ± 6.26	10.84 ± 7.01	10.43 ± 5.51	*0.697* [(a)]
Calcium (mg/kg)	5.16 ± 4.31	5.56 ± 4.17	6.28 ± 7.38	*0.676* [(a)]
Selenium (µg/kg)	1.12 ± 0.79	1.07 ± 0.76	1.07 ± 0.38	*0.913* [(a)]
Zinc (mg/kg)	0.12 ± 0.08	0.11 ± 0.08	0.11 ± 0.07	*0.970* [(a)]
Vit. A (µg/kg)	6.93 ± 5.66	8.77 ± 18.51	5.86 ± 7.60	*0.115* [(a)]
Vit. B1 (µg/kg)	0.02 ± 0.007	0.02 ± 0.01	0.02 ± 0.01	*0.215* [(a)]
Vit. B2 (µg/kg)	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.008	*0.282* [(a)]
Vit. B3 (µg/kg)	0.23 ± 0.16	0.21 ± 0.16	0.24 ± 0.13	*0.352* [(a)]
Vit. B6 (µg/kg)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	*0.552* [(a)]
Vit. B9 (µg/kg)	2.34 ± 1.79	2.23 ± 1.74	2.23 ± 1.76	*0.986* [(a)]
Vit. B12 (µg/kg)	0.05 ± 0.04	0.05 ± 0.06	0.04 ± 0.03	*0.396* [(a)]
Vit. C (µg/kg)	0.93 ± 0.75	0.81 ± 0.86	0.71 ± 0.92	*0.312* [(b)]
Vit. D (µg/kg)	0.04 ± 0.06	0.05 ± 0.13	0.04 ± 0.14	*0.246* [(a)]
Vit. E (µg/kg)	0.10 ± 0.05	0.12 ± 0.10	0.12 ± 0.09	*0.762* [(a)]

Source: from author (2021).

Subtitles: Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). †Data obtained from a 24-hour dietary recall interview. [#] Data adjusted by body mass. Values expressed as the mean ± standard deviation. P values represent the result of [(a)] Kruskal-Wallis one-way ANOVA on Ranks followed by Student-Newman-Keuls *post-hoc* test, and [(b)] One-way ANOVA followed by Student-Newman-Keuls *post-hoc* test.

As shown in Table 5, the biochemical analysis indicated an increase in urea (before and after hemodialysis) and creatinine circulating levels in the 6 and 12 month evaluations compared to the first evaluation (1 month) ($P<0.05$). Serum albumin was reduced in the 12 month evaluation compared to the 6 month evaluation ($P<0.05$). In addition, total proteins were higher and alkaline phosphatase was reduced in the 6 month evaluation compared to 1 months evaluation ($P<0.05$).

Table 5 - Biochemical parameters and dialysis quality in patients with chronic end-stage renal disease undergoing hemodialysis in a 12-month follow-up.

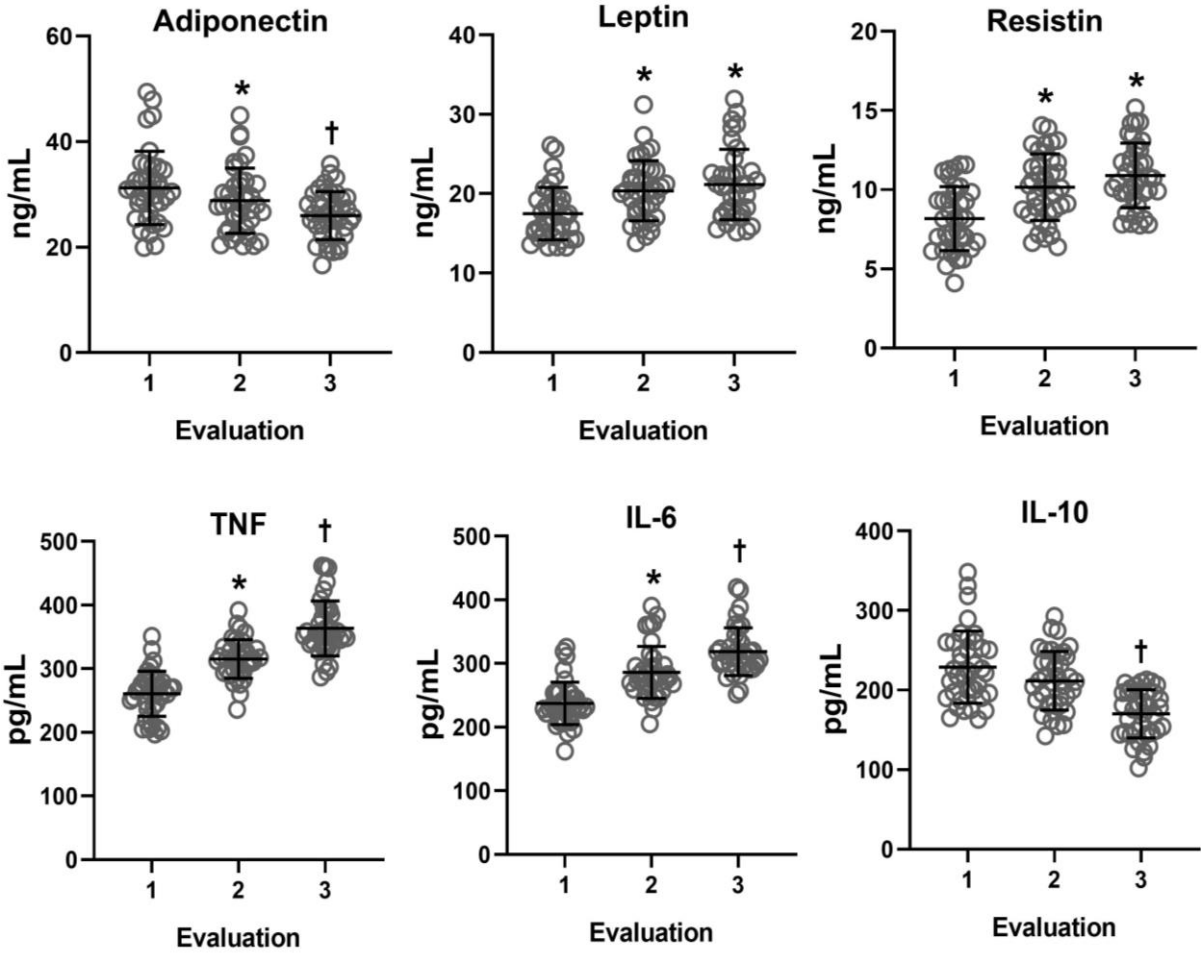
Variables	Evaluation 1	Evaluation 2	Evaluation 3	*P value*
Urea pre-dialysis (mg/dL)	83.84 ± 19.85	105.40 ± 32.18†	112.13 ± 35.30†	***<0.05***$^{(a)}$
Urea post-dialysis (mg/dL)	23.41 ± 10.81	36.01 ± 24.38†	37.26 ± 19.20†	***<0.05***$^{(a)}$
Creatinine (mg/dL)	8.18 ± 2.39	13.81 ± 14.84†	14.60 ± 12.29†	***<0.05***$^{(a)}$
Urea reduction rate (%)	71.87 ± 11.92	67.75 ± 13.41	67.46 ± 9.46	*0.157*$^{(b)}$
KT/V, n (%) <1.2	14 (36.84)	12 (31.58)	6 (15.79)	*0.104*$^{(c)}$
≥1.2	24 (63.16)	26 (68.42)	32 (84.21)	
Hemoglobin (g/dL)	11.35 ± 2.13	11.04 ± 2.23	11.74 ± 2.46	*0.448*$^{(a)}$
Hematocrit (%)	44.3 ± 55.24	34.57 ± 6.46	35.69 ± 5.88	*0.691*$^{(a)}$
Leucocytes × 10^2	62.88 ± 21.21	65.40 ± 17.33	62.23 ± 18.78	*0.781*$^{(b)}$
Platelets × 10^3	204.08 ± 55.66	190.66 ± 64.86	192.24 ± 54.37	*0.480*$^{(a)}$
Potassium (mg/dL)	5.38 ± 0.71	5.85 ± 0.88	6.97 ± 8.07	*0.087*$^{(b)}$
Calcium (mg/dL)	7.87 ± 0.98	8.56 ± 1.09	9.67 ± 1.47	*<0.095*$^{(a)}$
Phosphorus (mg/dL)	7.73 ± 1.41	7.17 ± 3.09†	8.21 ± 8.31	*<0.115*$^{(a)}$
Serum iron (mg/dL)	53.42 ± 24.99	64.03 ± 30.47	62.52 ± 30.58	*0.180*$^{(b)}$
Ferritin (ng/mL)	329.27 ± 327.95	304.66 ± 322.55	474.23 ± 1093.45	*0.877*$^{(a)}$
Blood glucose (mg/dL)	129.95 ± 61.30	146.53 ± 74.72	132.47 ± 56.62	*0.189*$^{(b)}$
Total cholesterol (mg/dL)	143.24 ± 27.03	148.35 ± 25.48	151.05 ± 28.02	*0.171*$^{(b)}$
HDL cholesterol (mg/dL)	42.27 ± 8.09	46.59 ± 13.11	51.22 ± 56.15	*0.163*$^{(b)}$
Triacylglycerol (mg/dL)	120.66 ± 64.10	123.07 ± 50.29	130.38 ± 62.22	*0.178*$^{(b)}$
Total proteins (mg/dL)	6.71 ± 0.61	7.13 ± 0.84†	6.93 ± 0.56	***<0.05***$^{(a)}$
Albumin (mg/dL)	3.73 ± 0.22	3.86 ± 0.44	3.61 ± 0.21‡	***<0.05***$^{(a)}$
PTH (pg/mL)	718.05 ± 751.52	616.59 ± 520.41	504.81 ± 517.87	*0.265*$^{(a)}$
TSI	22.43 ± 11.22	23.87 ± 9.97	130.94 ± 470.16	*0.718*$^{(a)}$

Source: from author (2021).

Subtitles: Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). *Kt/V*: *K* = urea clearance dialyzer; *t* = treatment time; *V* = volume of distribution of urea; PTH = parathyroid hormone; TSI = transferrin saturation index. P values represent the result of $^{(a)}$ Kruskal-Wallis one-way ANOVA on Ranks followed by Student-Newman-Keuls *post hoc* test, $^{(b)}$ One-way ANOVA followed by Student-Newman-Keuls *post hoc* test, and $^{(c)}$ Fisher exact test. Statistical difference (P<0.05) compared to †Evaluation 1, ‡ Evaluation 2.

As shown in Fig. 1, the results of the immunological assays indicated that adipocytes and cytokines exhibited a marked variability compared to nutritional and biochemical parameters. Thus, adiponectin serum levels was reduced (P<0.05) while leptin, resistin, TNF, and IL-6 were increased (P<0.05) in 6 and 12 months evaluation compared to 1 months evaluation (P<0.05). Adiponectin and IL-10 were reduced while TNF and IL-6 were increased in 12 months evaluation compared to 1 and 6 months evaluation (P<0.05).

Figure 1 - Adipokines and cytokine serum levels in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.



Source: From author (2021).

Subtitles: Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). Values expressed as the mean and standard deviation. * † The symbols indicate statistical difference ($P \leq 0.05$) compared to * Evaluation 1, † Evaluation 1 and 2 (one-way ANOVA followed by Student-Newman-Keuls *post-hoc* test).

As indicated in Table 6, adiponectin exhibited positive and significant correlation with fat mass (FM) in 1 month evaluation (P<0.05), which was not maintained in subsequent evaluations. Conversely, correlation between FM and leptin circulating levels was stable in all

evaluation (1, 6 and 12 months) (P<0.05). Resistin and FM presented no significant correlation in all evaluations (P>0.05). In addition, no significant correlation between adipokines (adiponectin, leptin and resistin), creatinine and urea pre-dialysis was identified in all time-points analyzed (P>0.05).

Table 6 - Correlation between adipokines, fat mass, creatinine and urea circulating levels in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Adipokines (ng/mL)		Evaluation 1		Evaluation 2		Evaluation 3	
		R	P value	R	P value	R	P value
Adiponectin	× FM (%)	-0.366	***0.023***	-0.146	*0.382*	*0.176*	*0.290*
	× Creatinine (g/dL)	0.160	*0.336*	0.082	*0.622*	*0.153*	*0.419*
	× Urea pre (g/dL)	0.114	*0.497*	0.099	*0.553*	*0.181*	*0.276*
Leptin	× FM (%)	0.693	***<0.001***	0.514	***<0.001***	*0.405*	***0.011***
	× Creatinine (g/dL)	0.034	*0.837*	0.219	*0.185*	*0.189*	*0.256*
	× Urea pre (g/dL)	0.289	*0.078*	0.224	*0.176*	*0.152*	*0.361*
Resistin	× FM (%)	0.190	*0.253*	0.285	*0.083*	*0.255*	*0.122*
	× Creatinine (g/dL)	0.277	*0.092*	0.103	*0.539*	*0.126*	*0.451*
	× Urea pre (g/dL)	0.248	*0.133*	0.141	*0.339*	*0.156*	*0.349*

Source: from author (2021).

Subtitles: FM: Fat mass (%), Urea pre: Urea pre-dialysis, R: correlation coefficient. Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). P values in bold indicate significant correlation from Pearson test ($P \leq 0.05$).

The results of linear regression for adipokines as dependent variables are shown in Table 7. Reinforcing the correlation results, our linear regression models indicated that 16.3 to 47.9% leptin variability were explained by the relative (%) FM distribution (P<0.05). Creatinine and pre-dialysis urea were unable to explain leptin variability (P>0.05). In addition, FM, creatinine and pre-dialysis urea presented no predictive significance on adiponectin and resistin circulating levels (P>0.05).

Table 7 - Linear regression models with adipokines as dependent variables according fat mass, creatinine and urea circulating levels in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Adipokines (ng/mL)		Evaluation 1			Evaluation 2			Evaluation 3		
		β	R2	*P*	*β*	R2	*P*	*β*	R2	*P*
Adiponectin	× FM (%)	*-2.139*	*0.134*	*0.023*	*-4.751*	*0.021*	*0.381*	*3.141*	*0.031*	*0.290*
	× Creatinine (g/dL)	*25.91*	*0.012*	*0.496*	*3.010*	*0.006*	*0.6218*	*10.91*	*0.018*	*0.419*
	× Urea pre (g/dL)	*2.213*	*0.025*	*0.336*	*1.933*	*0.009*	*0.553*	*0.710*	*0.032*	*0.275*
Leptin	× FM (%)	*0.537*	*0.479*	***<0.001***	*0.826*	*0.264*	***0.001***	*1.332*	*0.163*	***0.011***
	× Creatinine (g/dL)	*40.58*	*0.001*	*0.837*	*0.694*	*0.048*	*0.185*	*7.598*	*0.035*	*0.255*
	× Urea pre (g/dL)	*0.582*	*0.083*	*0.078*	*0.525*	*0.050*	*0.176*	*0.852*	*0.023*	*0.361*
Resistin	× FM (%)	*1.195*	*0.036*	*0.252*	*0.830*	*0.080*	*0.083*	*0.974*	*0.064*	*0.122*
	× Creatinine (g/dL)	*3.092*	*0.076*	*0.092*	*0.823*	*0.010*	*0.538*	*5.254*	*0.015*	*0.451*
	× Urea pre (g/dL)	*0.414*	*0.061*	*0.132*	*0.464*	*0.019*	*0.398*	*0.370*	*0.024*	*0.349*

Source: from author (2021).

Subtitles: FM: Fat mass (%), Urea pre: Urea pre-dialysis. *P* values in bold indicate statistical significance for individual predictors in the regression models (P≤0.05). *Equations with significant result obtained from linear regression analysis in Evaluation 1: Leptin (ng/mL) = 10.881 + (0.258 × %Fat mass), Evaluation 2: Leptin (ng/mL) = 14.605 + (0.219 × %Fat mass), Evaluation 3: Leptin (ng/mL) = 15.315 + (0.218 × %Fat mass).

As indicated in Table 8, TNF and IL-6 exhibited positive and significant correlation with creatinine and pre-dialysis urea at 1, 6 and 12 months evaluation (P<0.05). Conversely, IL-10 circulating levels was inversely and significantly correlated with creatinine and pre-dialysis urea at 1, 6 and 12 months evaluation (P<0.05). TNF, IL-6 and IL-10 serum levels exhibited no significant correlation with FM in all time-points analyzed (P>0.05).

Table 8 - Correlation between cytokines, fat mass, creatinine and urea circulating levels in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Cytokines (pg/mL)		Evaluation 1		Evaluation 2		Evaluation 3	
		R	P value	R	P value	R	P value
TNF	× FM (%)	*0.140*	*0.401*	*0.248*	*0.132*	*0.129*	*0.439*
	× Creatinine (g/dL)	*0.394*	***0.014***	*0.484*	***0.002***	*0.599*	***<0.001***
	× Urea pre (g/dL)	*0.444*	***0.005***	*0.681*	***<0.001***	*0.684*	***<0.001***
IL-6	× FM (%)	*0.232*	*0.161*	*0.136*	*0.415*	*0.232*	*0.161*
	× Creatinine (g/dL)	*0.419*	***0.008***	*0.467*	***0.003***	*0.458*	***0.003***
	× Urea pre (g/dL)	*0.520*	***<0.001***	*0.638*	***<0.001***	*0.543*	***<0.001***
IL-10	× FM (%)	*0.238*	*0.150*	*0.123*	*0.463*	*0.0692*	*0.680*
	× Creatinine (g/dL)	*-0.405*	***0.011***	*-0.401*	***0.012***	*-0.475*	***0.002***
	× Urea pre (g/dL)	*-0.527*	***<0.001***	*-0.511*	***0.001***	*-0.562*	***<0.001***

Source: from author (2021).

Subtitles: TNF: Tumor necrosis factor, IL: Interleukin, FM: Fat mass (%), Urea pre: Urea pre-dialysis, R: correlation coefficient. Evaluations: 1 (1 month), 2 (6 months), and 3 (12 months). *P values in bold indicate significant correlation from Pearson test ($P \leq 0.05$).

The results of linear regression for cytokines as dependent variables are shown in Table 9. In line with the correlation results, our linear regression models indicated that creatinine and urea pre-dialysis, but no FM, exhibited predictive relevance to explain the variability in TNF, IL-6 and IL-10 circulating levels in all time-points analyzed. In this sense, creatinine and urea pre-dialysis respectively explained 15.5 to 35.9% and 19.6 to 47.7% TNF, 17.5 to 21.8% and 27.0 to 40.6% IL-6, as well as 16.0 to 22.5% and 26.0 to 31.6% IL-10 variability at 1, 6 and 12 months evaluation.

Table 9 - Linear regression models with cytokines as dependent variables according fat mass, creatinine and urea circulating levels in patients with chronic end-stage renal disease undergoing hemodialysis (n= 38) in a 12-month follow-up.

Cytokines (ng/mL)		Evaluation 1			Evaluation 2			Evaluation 3		
		β	R2	*P*	*β*	R2	*P*	*β*	R2	*P*
TNF	× FM (%)	*28.50*	*0.019*	*0.401*	*13.77*	*0.061*	*0.132*	*40.39*	*0.016*	*0.438*
	× Creatinine (g/dL)	*38.13*	*0.155*	***0.014***	*2.536*	*0.234*	***0.002***	*23.220*	*0.359*	***<0.001***
	× Urea pre (g/dL)	*4.081*	*0.196*	***0.005***	*1.390*	*0.463*	***0.001***	*1.781*	*0.467*	***<0.001***
IL-6	× FM (%)	*16.280*	*0.053*	*0.161*	*33.75*	*0.018*	*0.415*	*19.67*	*0.053*	*0.160*
	× Creatinine (g/dL)	*33.870*	*0.175*	***0.008***	*3.523*	*0.218*	***0.003***	*26.53*	*0.210*	***0.003***
	× Urea pre (g/dL)	*3.287*	*0.270*	***<0.01***	*1.992*	*0.406*	***<0.001***	*1.960*	*0.294*	***<0.001***
IL-10	× FM (%)	*21.48*	*0.056*	*0.150*	*33.74*	*0.015*	*0.462*	*53.41*	*0.004*	*0.679*
	× Creatinine (g/dL)	*-47.45*	*0.164*	***0.011***	*-3.708*	*0.160*	***0.012***	*-20.74*	*0.225*	***0.002***
	× Urea pre (g/dL)	*-4.389*	*0.278*	***<0.01***	*-2.247*	*0.260*	***<0.001***	*-1.533*	*0.316*	***<0.001***

Source: from author (2021).

Subtitles: TNF: Tumor necrosis factor, IL: Interleukin, FM: Fat mass (%), Urea pre: Urea pre-dialysis. *P* values in bold indicate statistical significance for individual predictors in the regression models (P≤0.05). *Equations with significant result (P<0.05) obtained from multiple linear regression analysis: *Equations with significant result obtained from multiple linear regression analysis in Evaluation 1: TNF (pg/mL) = 182.527 + (0.594 × Urea) + (3.452 × Creatinine). IL-6 (pg/mL) = 147.129 + (0.719 × Urea) + (3.646 × Creatinine). IL-10 (pg/mL) = 346.858 - (1.002 × Urea) - (4.169 × Creatinine). Evaluation 2: TNF (pg/mL) = 239.877 + (0.638 × Urea) + (0.582 × Creatinine). IL-6 (pg/mL) = 203.508 + (0.709 × Urea) + (0.550 × Creatinine). IL-10 (pg/mL) = 281.161 - (0.581 × Urea) - (0.589 × Creatinine). Evaluation 3: TNF (pg/mL) = 262.870 + (0.626 × Urea) + (3.495 × Creatinine). IL-6 (pg/mL) = 248.803 + (0.452 × Urea) + (2.192 × Creatinine). IL-10 (pg/mL) = 232.341 - (0.372 × Urea) - (2.357 × Creatinine).

5 DISCUSSION

Hemodialysis patients are exposed to marked metabolic and pro-inflammatory overload, whose management requires strict control of the dialysis dose and nutritional readjustment (Kanno et al., 2021). Although monitoring these conditions is essential to offer a more efficient and personalized treatment, the relationship between variability in biochemical, nutritional and inflammatory indicators for hemodialysis patients remains overlooked. From sex stratification, the sample investigated presented similar characteristics of age, body mass, comorbidities, family history of kidney disease, and time in hemodialysis. In addition, men and women presented a low frequency of smoking and have not reported alcohol intake. Thus, the nutritional, biochemical and immunological outcomes of interest showed limited interference from the sample characteristics, reflecting a biological behavior predominantly related to the clinical condition of the evaluated patients. From the use of different and complementary assessment tools, we identified that hemodialysis patients had marked time-dependent stability in anthropometric and nutritional characteristics. There is consistent evidence that nutritional parameters (i.e., BMI, BAI, WC, WHR, fat and lean mass distribution, and profile of nutrient intake) are relevant markers of cardiovascular morbidity and mortality risk in the general population (Amirabdollahian and Haghighatdoost, 2018; Darbandi et al., 2020). However, these relationships are more complex and less predictable in hemodialysis patients, as they are aggravated by the chronic uremic-inflammatory syndrome in this population (Okuno, 2021). Considering the clinical monitoring of hemodialysis patients, less variability in these nutritional variables can favor the early identification of potential risk factors, which may result from the worsening of the uremic syndrome and the consequent decline in the patient's clinical condition (Ikizler, 2013; Okuno, 2021).

Classically, weight control is a central goal of the nutritional management in hemodialysis patients (Lim et al., 2019; Cupisti et al., 2020). Although obesity is a risk factor for chronic kidney disease (Amirabdollahian and Haghighatdoost, 2018; Darbandi et al., 2020), an obesity paradox is systematically reported in observational studies, in which higher BMI (> 10th percentile) IS protective and associated with greater survival in maintenance hemodialysis patients (Molnar et al., 2011; Park et al., 2014). However, obesity can represent a disadvantage for these patients, especially considering that BMI above 30 or 35 kg/m^2 may be an impediment to kidney transplantation (Molnar et al., 2011). Despite its predictive relevance for morbimortality, the isolated use of BMI as an obesity marker is not ideal, as it does not adequately reflect lean and fat mass distribution (Molnar et al., 2011). Accordingly,

the combination of multiple markers is required to better delimit the cardiometabolic risk of the hemodialysis patient (Amirabdollahian and Haghighatdoost, 2018; Segall et al., 2014). From this comprehensive assessment, we identified that the variability in malnutrition risk was clearly influenced by the tool/marker used. However, the nutritional status was consistent over time, demonstrating low variability for the same measurement instrument. In this sense, all the tools used showed a significant risk of malnutrition in all time-points investigated, in line with the high prevalence (40% to 70%) of malnutrition in hemodialysis patients (Akhlaghi et al., 2021; Cohen-Hagai et al., 2020). The risk of malnutrition was especially detected from the modified subjective global assessment, which has been consistently designed and applied to hemodialysis patients (Vegine et al., 2011; Silva et al., 2019). Taken together, these findings are in line with the evidence that malnutrition is more of a rule than an exception in hemodialysis patients (Kistler et al., 2018; Silva et al., 2019). Unfortunately, this condition is not surprising, especially considering the nutritional repercussions of the dramatic metabolic imbalance caused by the uremic syndrome, which include anorexia, vomiting, indigestion, and severe protein catabolism (Kistler et al., 2018).

Although the stability in nutritional variables observed in this study favors the early detection of cardiometabolic risk, this stable behavior also brings to light the evident limitation in improving the nutritional status of malnourished hemodialysis patients. Accordingly, despite receiving weekly nutritional monitoring, most patients with some degree of malnutrition maintained their nutritional status throughout the 12-month follow-up. This is an important characteristic, which indicates a marked difference between hemodialysis patients compared to the general population, whose nutritional deficiencies are best controlled through dietary manipulation (Lim et al., 2019; Cupisti et al., 2020). In addition, we assessed the nutrient intake profile of all patients during nutritional monitoring and identified a similarly stable nutrient intake at all investigated times. This finding indicates an important adherence to the planned diet for hemodialysis patients, a result potentially associated with an adequate nutritional monitoring provided in the hemodialysis center. There is no doubt that nutritional therapy is an concurrent challenge in the management of hemodialysis patients, since in addition to eating disorders, hemodialysis treatment demands strict control of nutrients intake, especially a severe restriction on water, electrolytes and proteins intake (Kistler et al., 2018). Thus, dietary interventions must be carefully tailored to not provide additional metabolic load and to avoid the worsening of the catabolic state typically perceived in this population (Cupisti et al., 2020).

Despite the stability in cardiometabolic markers and nutrient intake, hemodialysis patients showed remarkable biochemical variability in urea, creatinine, total proteins, and albumin circulating levels. Interestingly, all these parameters are directly involved in protein metabolism, which is severely compromised in hemodialysis patients (Nakazato et al., 2015; Silva et al., 2018). In this sense, our findings reinforce the relevance of these parameters as markers of protein metabolism, allowing us to improve the interpretation of anthropometric measurements used to estimate potential muscle mass losses (Ikizler et al., 2013; Patel et al., 2013). Accordingly, increased urea and creatinine levels are directly correlated to muscle protein catabolism, whose kinetics is profoundly influenced by dialysis clearance, nutritional status, protein intake, and hydration (Patel et al., 2013). In addition, urea and creatinine are markers systematically incorporated into the evaluation of protein-energy wasting (PEW), which is a persistent depletion of protein/energy stores with high prevalence (50% to 70%) and closed correlated to increased morbidity and mortality in hemodialysis patients (Sabatino et al., 2017). Protein-energy wasting also exerts negative impact on blood proteins such as albumin, whose levels in hemodialysis patients may be lower than in the general population in response to reduced dietary protein intake, loss of amino acids in the dialysate, resistance to anabolic hormones, and inflammation (Segall et al., 2014; van Gelder et al., 2018). Thus, the higher variability in albumin levels reinforce the evidence that this protein requires a rigorous monitoring, especially considering that hypoalbuminemia is a consistent predictor of cardiovascular diseases and all-cause mortality in hemodialysis patients (van Gelder et al., 2018).

In addition to nutritional and biochemical disturbances, uremic syndrome triggers a chronic systemic inflammatory process that should be carefully monitored, since malnutrition and inflammation act as cumulative risk factors for cardiovascular diseases and death in ESRD patients (Stenvinkel et al., 1999). Accordingly, we identified marked variability in all investigated adipokines and cytokines during the 12-month follow-up. Interestingly, time-dependent increase in TNF, IL-6, leptin and resistin levels, as well as reduction in adiponectin and IL-10 were accompanied by a reciprocal increase in urea and creatinine levels. However, only leptin exhibited a consistent and direct time-dependent correlation with relative fat mass, which presented a limited predictive relevance on leptin results. Conversely, creatinine and pre-dialysis urea were directly correlated with TNF and IL-6, and inversely correlated with IL-10 circulating levels. In addition, creatinine and pre-dialysis urea presented predictive relevance for these cytokines in all time-points investigated, reinforcing the proposition of a potential dependence between uremic overload and inflammatory stress (Cohen and

Narayanan, 2019), and that routine biochemical markers may be relevant to estimate the inflammatory status in hemodialysis patients (Cobo et al., 2018).

Uremic-inflammatory syndrome is currently admitted as a major determinant of the increased mortality risk in hemodialysis patients (100 to 200 times higher) compared to the general population (Markaki et al., 2016; Castillo-Rodríguez et al., 2017). Although the pathogenesis of this syndrome is not fully understood, the imbalance in anti- and pro-inflammatory mediators derives from the cumulative effect of uremic toxins, dialysis characteristics (i.e., catheter infection, dialysis fluid leakage, and biological incompatibility of the dialysis membrane), and comorbidities (Cobo et al., 2018; Chen et al., 2019). Thus, hemodialysis patients experience a dramatic modulation of several immunological effectors, especially adipokines and cytokines (Akchurin and Kaskel, 2015; Castillo-Rodríguez et al., 2017). These molecules orchestrate the chronic systemic inflammatory syndrome, modulating the clinical outcomes in hemodialysis patients according to their specific biological role (Stenvinkel et al., 2005; Lim et al., 2015).

Previous evidence indicate that increased leptin and resistin are influenced by TNF and IL-6 signaling pathway, which are often upregulated in uremic patients and exert a profound metabolic impact by regulating food intake, hormone biosynthesis, angiogenesis lipid and glucose kinetics (Stenvinkel et al., 2005; Raman and Khanal 2021). Combined, these changes act as direct nutritional and cardiometabolic risk factors, which are associated with dyslipidemia, cardiovascular diseases, and increased mortality in hemodialysis patients (Stenvinkel et al., 2005; Raman and Khanal, 2021). In this sense, the sustained increase in leptin levels is considered as independent risk factor for acute cardiovascular events, since predisposes to NO downregulation, atherogenesis (Raman and Khanal, 2021), platelets dysfunction, and hypertrophy of vascular smooth muscle cells (Wolley and Hutchison, 2018). Cardiovascular diseases are also associated to high resistin levels in ESRD patients (Vahdat, 2018), which is used as a marker of increased risk for the occurrence of heart failure and sudden death (Zhang et al., 2011). Interestingly, preclinical studies of kidney disease indicated that leptin and resistin effects are antagonized by adiponectin, which exerts anti-atherogenic, anti-inflammatory, and cardiovascular protective effects (Teta, 2012). However, the uremic-inflammatory microenvironment may attenuate adiponectin biosynthesis, aggravating metabolic syndrome and cardiovascular risk in hemodialysis patients (Teta, 2012; Vahdat, 2018).

In addition to adipokines, Th1 cytokines such as IL-6 and TNF exerts a major impact on the chronic inflammatory syndrome implicated in cardiovascular outcomes in ESRD patients

(Castillo-Rodríguez et al., 2017). Thus, a 4- to 5-fold increase in IL-6 and TNF circulating levels is reported in hemodialysis patients, playing a direct role in the development of cardiomyocytes contractile dysfunction, myocardial fibrosis, atherosclerotic lesions and thromboembolic events, orchestrating the recruitment of inflammatory cells and procoagulant mechanisms (Stenvinkel et al., 2005; Hartman and Frishman, 2014; Castillo-Rodríguez et al., 2017). Accordingly, IL-6 and TNF are considered the greatest predictors of cardiovascular disease and all-cause mortality in hemodialysis patients (Kleinbongard et al., 2010; Hartman and Frishman, 2014; Castillo-Rodríguez et al., 2017), reinforcing the implication of these cytokine as relevant immunological markers in this population. In addition, IL-10 is a Treg cytokine with potent anti-inflammatory properties known to antagonize pro-inflammatory mechanisms triggered by pro-inflammatory effectors, including IL-6 and TNF. However, although IL-10 exerts cardioprotective effects, this cytokine is unable to control high-grade inflammation in uremic patients (Stenvinkel et al., 2005; Chen et al., 2021), reinforcing the link between uremic overload and pro-inflammatory stress. Interestingly, the IL-10 gene polymorphism (i.e., -1082 A allele) was previously detected in hemodialysis patients, a condition associated to reduced IL-10 production with a notorious predictive relevance for cardiovascular mortality in this population (Girndt et al., 2002). Thus, monitoring IL-10 levels has a dual purpose, being relevant as a predictor of mortality and as a marker of immunological balance in hemodialysis patients, which takes into account the relationship between pro- and anti-inflammatory mediators.

6 CONCLUSIONS

From a comprehensive longitudinal screening, we identified that hemodialysis patients under clinical and nutritional monitoring exhibiting a reduced time-dependent variability in anthropometric/cardiometabolic risk factors and nutrients intake. However, biochemical markers (i.e., urea, creatinine, and serum proteins) and especially immunological effectors such as adipokines and cytokines presented a marked time-dependent variability. Leptin but not adiponectin and resistin levels was correlated and partially predicted from relative fat mass distribution in all time-points analyzed. Although this relationship has not been observed for cytokines (TNF, IL-6, and IL-10), these molecules showed a closed correlation and were consistently predicted from creatinine and pre-dialysis urea levels in a 12-month follow-up, indicating a potential relationship between uremic and inflammatory stress.

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ANNEX A - Financial support

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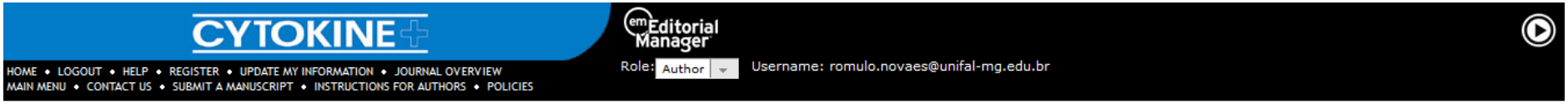
ANNEX B - Manuscript submitted



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Action	Manuscript Number	Title	Initial Date Submitted	Status Date	Current Status
Action Links	CYTO-21-712	Relationship between time-dependent variability in cardiometabolic risk factors and biochemical markers with cytokine and adipokine levels in hemodialysis patients	2021-08-19 17:35:40	2021-10-04 11:56:05	Under Review

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Cytokine

Relationship between time-dependent variability in cardiometabolic risk factors and biochemical markers with cytokine and adipokine levels in hemodialysis patients

--Manuscript Draft--

Manuscript Number:	CYTO-21-712
Article Type:	Full length article
Keywords:	Malnutrition; hemodialysis; inflammation; kidney disease
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