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PAULA CORRÊA SILVEIRA DA SILVA

RELATIONSHIP BETWEEN ADVANCED GLYCOSYLATION END PRODUCTS, INFLAMMATION AND OXIDATIVE STRESS IN PATIENTS WITH END-STAGE RENAL DISEASE UNDERGOING HEMODIALYSIS

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Tese apresentada como parte dos requisitos para obtenção do título de Doutora em Biociências Aplicadas à Saúde pela Universidade Federal de Alfenas.
Área de concentração: Neurociências e comportamento.

Orientador: Prof. Dr. Rômulo Dias Novaes

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Aprovada em: 15 de fevereiro de 2023

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RESUMO

O controle de efetores pró-inflamatórios e pró-oxidantes sistêmicos é essencial para mitigar o risco cardiovascular e a mortalidade em pacientes com doença renal terminal (ESRD). No entanto, monitorar esses processos ainda é desafiador devido à alta incerteza sobre seus determinantes e preditores sensíveis e específicos. Assim, investigamos a relação entre produtos finais de glicosilação avançada (AGE), estado pró-inflamatório e pró-oxidante em pacientes com doença renal terminal (DRT) em hemodiálise (HD). Além do perfil nutricional e da eficiência de diálise, AGE, citocinas, quimiocinas, proteína C reativa (PCR), capacidade antioxidante total (TAC) e não proteica (npAC), oxidação lipídica e proteica foram analisados em amostras de sangue de 49 pacientes em HD. AGE, PCR, citocinas (TNF, IL-6 e IL-10), quimiocinas (CCL2, MIP-1α e MIP-1B), proteínas carbonildas e malondialdeído foram regulados positivamente, enquanto TAC e npAC foram regulados negativamente em pacientes em HD comparação com indivíduos de saúde. A eficiência da diálise foi reduzida, enquanto a contagem de leucócitos, uréia pré- e pós-HD foram aumentadas em pacientes com maior acúmulo de AGE. Os níveis séricos de PCR, proteínas carboniladas, malondialdeído e todas as citocinas e quimiocinas analisadas foram correlacionados com os níveis circulantes de AGE. Os níveis de AGE foram inversamente correlacionados com IL-10, TAC e npAC em pacientes com maior acúmulo de AGE. Além disso, a AGE apresentou valor preditivo acentuado (coeficiente de determinação) para explicar a variabilidade da PCR, citocinas, quimiocinas, proteínas carbonildas, malondialdeído, TAC e npAC em pacientes com níveis mais elevados de AGE. Em conjunto, nossos achados fornecem evidências de que o acúmulo de AGE está associado a importantes efetores pró-inflamatórios e pró oxidantes em pacientes com DRT submetidos à HD. Assim, o monitoramento de AGE pode ser relevante para predizer o estresse inflamatório sistêmico e o equilíbrio entre o estado oxidante e antioxidante nesses pacientes.

Palavras-chave: Preditores biológicos; Hemodiálise; Inflamação; Estresse oxidativo.

ABSTRACT

Controlling systemic proinflammatory and prooxidant effectors is essential for mitigating cardiovascular risk and mortality in patients with end-stage renal disease (ESRD). However, monitoring these processes is still challenging due to the high uncertainty about their determinants and predictors. Thus, we investigated the relationship between advanced glycosylation end products (AGE), proinflammatory and prooxidant status in ESRD patients undergoing hemodialysis (HD). In addition to nutritional profile and dialysis efficiency, AGE, cytokines, chemokines, C-reactive protein (CRP), total antioxidant capacity (TAC) and antioxidant capacity non-protein (npAC), lipid and protein oxidation were analyzed in blood samples from 49 HD patients. AGE, CRP, cytokines (TNF, IL-6 and IL-10), chemokines (CCL-2, MIP-1α and MIP-1B), protein carbonyl, and malondialdehyde were upregulated, while TAC and npAC were down-regulated in HD patients compared to heath subjects. Dialysis efficiency was reduced, while leukocytes counting, pre- and post-HD urea were increased in patients with higher AGE accumulation. Serum levels of CRP, protein carbonyl, and malondialdehyde and all cytokines and chemokines analyzed were correlated with AGE circulating levels. AGE was inversely correlated with IL-10, TAC and npAC in patients with higher AGE accumulation. AGE exhibited marked predictive value (determination coefficient) to explain CRP, cytokines, chemokines, PCN, MDA, TAC and npAC variability in patients with higher AGE levels. Taken together, our findings provide evidence that ACE accumulation is associated with important proinflammatory and prooxidant effectors in patients with ESRD undergoing hemodialysis. Thus, ACE monitoring may be relevant to predict systemic inflammatory stress and the balance between oxidant and antioxidant status in these patients.

Keywords: Biological predictors; Hemodialysis; Inflammation; Oxidative stress.

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LIST OF ACRONYMS

AGE	Advanced glycosylation end products
CHF	Congestive heart failure
CKD	Chronic kidney disease
CNT	Control volunteers
CRP	C-reactive protein
CVD	Cardiovascular diseases
DM	Diabetes mellitus
DRT	Doença renal terminal
ESRD	End-stage renal disease
GOA	Global objective assessment
HD	Hemodialysis
KD	Kidney disease
MDA	Malondialdehyde
npAC	Antioxidant capacity non-protein
PCN	Protein carbonyl
SAH	Systemic arterial hypertension
TAC	Total antioxidant capacity
TSI	Transferrin saturation index

SUMMARY

1 INTRODUCTION

Chronic kidney disease (CKD) is a destabilizing condition with complex and multifactorial etiology, which is closely correlated to metabolic risk factors such as inflammatory diseases, diabetes mellitus and systemic arterial hypertension (Kalantar-Zadeh et al., 2021; Modi et al., 2019). Despite advances in controlling these comorbidities and refinements in renoprotective therapies, it is not always possible to prevent CKD progression to end-stage renal disease (ESRD - glomerular filtration rate <15 mL/min). At this stage, renal failure triggers uremic syndrome, which is characterized by intense retention of uremic toxins (e.g., urea, uric acid, cytokines, kynurenic acid, and p-cresylsulfate) that would normally be excreted (Duranton et al., 2012; Stinghen et al., 2016). These toxins trigger a complex pathophysiological process consistently associated with the development of systemic inflammation, cardiovascular diseases (CVD) and increased mortality risk in ESRD patients (Dai et al., 2017; Modi et al., 2019).

Uremic syndrome shifts systemic metabolism towards a prooxidant and proinflammatory pathological phenotype (Cobo et al., 2018; Duranton et al., 2012). Accordingly, systemic inflammation and redox imbalance interact in an synergistic way, determining severe nutritional disorders (e.g., absorptive deficiency and sarcopenia), marked catabolic status and metabolic fragility (e.g., anemia, proteasome upregulation, and protein-energy wasting) in ESRD patients, especially those undergoing maintenance hemodialysis (HD) (Carrera-Jiménez et al., 2018; Silva et al., 2019). Due to the central role of chronic inflammation and oxidative stress in CVD development and progression, monitoring these events has been indicated to estimate metabolic stress magnitude, treatment efficacy and risk of death in HD patients (Ribeiro et al., 2022; Silva et al., 2019; Silva et al., 2021). However, this monitoring is still limited, since the pathogenesis of inflammation and redox imbalance is not yet fully understood, and especially due to the limited availability of viable (economically and technically) and accurate predictive markers.

Biosynthesis and accumulation of advanced glycosylation end products (AGEs) has been suggested as a mechanism underlying inflammation and oxidative stress in HD patients (Jiang et al., 2021; Perkins et al., 2021; Stinghen et al., 2016). However, the predictive relevance of AGEs in estimating the levels of central inflammatory (e.g., TNF, IL-6, and CCL2) and oxidative stress (e.g. free radicals and

malondialdehyde) effectors remains overlooked. AGEs are heterogeneous uremic toxins produced by non-enzymatic reactions between sugars or reactive α-dicarbonyls with positively charged amino groups of nucleic acids, lipids and proteins (Jiang et al., 2021; Perkins et al., 2021). AGEs activate nuclear factor kappa B (NF-κB), mitogen-activated protein kinases, Jun N-terminal kinase, and nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, regulating gene transcription for adhesion molecules, proinflammatory cytokines and chemokines, as well as triggering reactive ROS biosynthesis (Stinghen et al., 2016). Thus, AGEs have been linked to mechanisms centrally involved in endothelial dysfunction, vasculitis, atherosclerosis, vascular calcification (Stinghen et al., 2016), and mortality (Jiang et al., 2021; Meerwaldt et al., 2005; Stinghen et al., 2016) in ESRD patients.

Although interventions capable of limiting inflammation and oxidative stress are modern proposals to control risk factors and cardiovascular morbidity (Dai et al., 2017; Malekmakan et al., 2020; Stinghen et al., 2016), clinical monitoring of these events still depends on identifying viable and relevant predictors of inflammation and redox imbalance in CKD patients, such as cytokines, chemokines, total and non-protein antioxidants, oxidized lipids and proteins; which are markers potentially useful to guide clinical management by estimating systemic inflammatory and oxidative status in patients with ESRD undergoing hemodialysis.

1.1 OBJECTIVES

1.1.1 General objectives

This hospital-based clinical study was designed to assess the predictive relevance of AGEs accumulation in predicts inflammatory and oxidative status markers in patients with ESRD undergoing hemodialysis.

1.1.2 Specific objectives

a) To compare clinical characteristics of ESRD patients undergoing hemodialysis according to sex;
b) To compare clinical characteristics of ESRD patients undergoing hemodialysis according to AGEs stratification;
c) To characterize general biochemical parameters of ESRD patients undergoing hemodialysis according to AGEs stratification;

d) To compare the circulating levels of inflammatory markers in ESRD patients undergoing hemodialysis and health volunteers;
e) To compare the circulating levels of oxidant and antioxidant markers in ESRD patients undergoing hemodialysis and health volunteers;
f) To evaluate potential correlations between AGEs with inflammatory, oxidant and antioxidant markers in ESRD patients undergoing hemodialysis.

2 PATIENTS AND METHODS

2.1 STUDY DESIGN AND CASUISTRY

We conducted a cross-sectional study with adult ESRD patients of both sexes undergoing HD in the Renal Replacement Therapy Center of the Alzira Velano University Hospital, Brazil. Initially forty-nine HD patients (24 women and 25 men) were admitted after signing the Informed Consent Form Patients' submitted to renal transplantation, change in dialysis modality in the last 3 months, hemodynamic and biochemical instability, newly implanted catheters, less than 6 months of HD treatment, cognitive impairment indicated by the Mini Mental State Examination (Folstein et al., 1975), history of neoplastic disease, and uncontrolled diabetes (Silva et al., 2019). Four women and two men received a kidney transplant, and two other men had newly implanted catheters and were therefore excluded. Thus, 43 HD volunteers (20 women and 23 men) were included in this study. All of the following tests were carried out in the analysis laboratory of the Hospital Alzira Velano.

2.2 ETHICAL APPROVAL

This prospective study was approved by the Hospital Ethics Committee for Human Research (protocol 1.767.706). All procedures were performed following the ethical principles for medical studies stated in the Declaration of Helsinki (WMA, 2001).

2.3 HEMODIALYSIS PROTOCOL AND ETHICAL APPROVAL

All patients received the standard HD treatment included in the hospital routine, which consisted of 3-4h HD sessions three times a week (Monday, Wednesday and Friday). The treatment protocol was based on a dialysate stream at 500 mL/min constant rate and blood flow ranging from 300-450 mL/min. High flux polysulfone membranes with bicarbonate-buffered dialysate combined with low flow polysulfone membranes were used.

2.4 CLINICAL DATA, NUTRITIONAL SCREENING AND DIALYSIS EFFICIENCY

All data were collected during the second weekly HD session .Thus, the influence a longer interdialytic period (weekend) on uremic stress, as well as the accumulation of dialysis load at the end of the week were avoided (Silva et al., 2019).

Clinical characteristics such as age, body mass, comorbidities, time in hemodialysis, smoking and alcohol consumption were recorded from medical records and confirmed with all volunteers. Nutritional evaluation was based on the body mass index (BMI = body mass [kg]/ height [m2]) (Ribeiro et al., 2022) and the global objective assessment (GOA) standardized for patients undergoing HD treatment (Silva et al., 2019). The dialysis efficiency was estimated according to HD dose, which was monitored using the Kt/V method calculated as follows: Kt/V = - ln (R - 0.008 × t) + (4 - 3.5 × R) 0.55 × UF / V; where R is the product of urea levels before dialysis divided by urea levels after dialysis, t is the dialysis duration (h), - ln is the natural logarithm negative, UF is the weight loss (kg) after dialysis, and V is the volume of urea distribution (L). Thus, Kt/V ≥ 1.2 indicated adequate dialysis dose (Lowrie and Teehan, 1983).

2.5 BLOOD COLLECTION AND BIOCHEMICAL ANALYSIS

Blood samples were directly collected from venous access using Gel SST II Advance Vacutainer tubes (Becton Dickinson, San Jose, CA, USA) in the absence of anticoagulant (Silva et al., 2021). Leucocytes were quantified in total blood samples using an automatic high-efficiency hematological analyzer and high-grade human reagents (Sysmex, XE-2100, Sao Paulo, SP, Brazil). The serum was collected after centrifuging the blood samples at 3800 ×g and 4 ºC for 15 minutes. Urea, creatinine, blood glucose, glycated hemoglobin, total iron, total iron-binding capacity (TIBC), total protein and albumin were analyzed in serum samples using the spectrophotometric method and commercial colorimetric biochemical kits (Invitro, Itabira, MG, Brazil) (Ribeiro et al., 2022). Serum iron and TIBC results were used to calculate transferrin saturation index (TSI, %) according to the formula TSI = (serum iron × 100%) / TIBC (Beilby et al., 1992).

2.6 ADVANCED GLYCATION END PRODUCTS IMMUNOASSAY

Total advanced glycation end products (AGE) were quantified in 50 µL serum samples using a commercial colorimetric kit and the manufacturer’s instructions (ABCAM, Cambridge, MA, USA). This immunoassay is based on an anti-AGE capture antibody and a horseradish peroxidase (HRP)-linked secondary detection antibody. The reactions were analyzed at 450 nm from a 96-wells microplate reader (Anthos Zenyth 200, Biochrom, Cambridge, UK). The quantity of AGE adduct in the

samples was determined by comparing its optical density with that of a known AGE-bovine serum albumin (BSA) standard curve. The assay was based on an AGE detection range of 0.36 µg/mL to 100 µg/mL was.

2.7 C-REACTIVE PROTEIN IMMUNOASSAY

Systemic inflammatory status in HD patients was investigated from C-reactive protein (CRP) serum levels (Heidari, 2013). This marker was quantified from a 96-wells colorimetric enzyme-linked immunosorbent assay (ELISA) kit, following the manufacture's instructions (Sigma-Aldrich, San Luis, MO, USA). The optical density of the reactions was monitored in a microplate reader at 450 nm (Anthos Zenyth 200, Biochrom, Cambridge, UK). The assay was based on a detection range of 34.29-25000 pg/mL.

2.8 CYTOKINES AND CHEMOKINES IMMUNOASSAY

The cytokines interleukin-6 (IL-6), interleukin-10 (IL-10), tumor necrosis factor (TNF), as well as the chemokines C-C motif ligand 2 (CCL2), macrophage inflammatory proteins 1α (MIP-1α) and 1β (MIP-1β) were additionally used as markers of systemic inflammation in HD patients (Pawlac et al., 2005). These molecules were quantified in serum samples from flow cytometry bead array (CBA). Commercial CBA kits for human cytokines and chemokines were used following the manufacturer's instructions (BD Biosciences, San Diego, CA, USA). All serum samples were analyzed in a FACSVerse flow cytometer and quantified using the FCAP 3.0 software (BD Biosciences, San Diego, CA, USA). Blood samples from 42 healthy volunteers (21 women and 21 men) with similar age (55.13 ± 16.11) and BMI (23.47 ± 6.82) were collected to estimate a cut-off point for cytokines and chemokines. Standard curves were obtained from recombinant cytokines and chemokines in a concentration ranging from 20 to 5000 pg/mL. The respectively lower limits for cytokines and chemokines detection were 2.6 to 18.9 pg/mL and 2 to 2.8 pg/mL.

2.9 PROTEIN CARBONYLS BIOCHEMICAL ASSAY

The circulating levels of oxidized proteins was estimated from the quantification of protein carbonyl (PCn) in serum samples according to the method described by Levine et al. (1990). For this, a biochemical method based on the

detection of labeled protein hydrazone derivatives from a reaction with 2,4-dinitrophenylhydrazine was used. The optical density of the reactions was monitored in a microplate reader at 370 nm (Anthos Zenyth 200, Biochrom, Cambridge, UK). Protein carbonyl content was measured admitting a molar absorption coefficient of 21,000 M-1 cm-1 , and was represented as nmol/mg protein-1 (Reznick and Packer, 1994). The bicinchoninic acid (BCA) method (Bainor et al., 2011) was used to quantify protein levels in the serum samples, which were used to normalize PCn results.

2.10 MALONDIALDEHYDE BIOCHEMICAL ASSAY

Circulating oxidized lipids were measured by quantifying malondialdehyde (MDA) serum levels by high-performance liquid chromatography (HPLC), following a standardized method (Brown and Kelly, 1996). Briefly, serum samples were exposed to a 250 mm × 4.6 mm i.d. VC-ODS RP18 column, which was prepared with 50:50 (v/v) phosphate buffer (25 mM, pH 6.5). The mobile phase was based on methanol, using a flow rate of 0.8 mL min−1. Malondialdehyde was submitted to fluorometric detection at 532 nm/553nm wavelengths (excitation/emission). For this, the HPLC system was coupled to a RF-10AXL detector (Shimadzu Scientific Instruments, Kyoto, Japan) to ensure the sensitivity for low MDA concentrations relative to (TBA)2 adduct. The same procedure was adopted for tetraethoxypropane, which was used to calibrate MDA-TBA peak (Silva et al., 2019).

2.11 TOTAL AND NON-PROTEIN ANTIOXIDANT CAPACITY ASSAY

Total and non-protein antioxidant defenses were measured in serum samples of all HD patients (Samouilidou et al., 2003). For such, a colorimetric biochemical kit and the manufacturer’s instructions (TAC Assay Kit, Sigma Aldrich, Milwaukee, USA) were used. In this method, the antioxidant activity evaluated considering Cu2+ conversion to Cu+ by proteins and non-protein small molecules. Small antioxidant molecules were differentiated from proteins by applying a chemical inhibitor to prevent Cu2+ reduction by antioxidants proteins. The antioxidant capacity was quantified by spectrophotometry at 570 nm wavelength. The results (mM) were obtained from a standard curve obtained using trolox as the reference antioxidant (Silva et al., 2019).

2.12 STATISTICAL METHOD

Patients were initially stratified by sex to investigate variability in clinical, nutritional, and dialysis dose data. Then, patients were stratified according to median AGE values into two groups: i) Low glycosylation (AGE median). For both stratification methods, absolute and relative categorical variables were compared by the Fisher’s exact tests and Pearson’s chisquared method. Continuous variables were expressed as mean and standard deviation, and data distribution was analyzed using the D'Agostino-Pearson method. Nonparametric data were compared by the Mann–Whitney U test, and parametric data were submitted to the Student's t-test. The association between AGE, prooxidant and inflammatory markers was investigated using the Pearson correlation coefficient and linear regression. Results with P value ≤0.05 indicated statistical difference in all tests.

3 RESULTS

The study sample was composed by HD patients ranging from 18 to 65 years of age (53.49% women and 46.51% men), and 66.4 ± 14.1 kg (mean body mass. Systemic arterial hypertension alone (77.6%) or combined with diabetes mellitus (14.0%) and congestive heart failure (26.5%) were the main comorbidities associated to ESRD. The frequency of smoking was low (6.1%) and the mean time in HD was 4.09 ± 2.77 years. Pre-dialysis urea concentration was low (20.9%) and none patients reported alcohol intake. Most patients (65.1%) presented adequate dialysis dose (KT/V ≥ 1.2) and some degree of malnutrition (95.4%). All these parameters were similar in men and women ($P<0.05$) (TABLE 1).

Table 1 - General characteristics of all hemodialysis patients stratified by sex

Variables	Total (n= 43)	Women (n= 20)	Men (n= 23)	*P value**
Age (years), mean ± S.D.	53.3 ± 15.2	54.4 ± 15.6	52.2 ± 14.7	*0.636[(a)]*
Body mass (kg), mean ± S.D.	67.4 ± 14.1	64.6 ± 15.1	70.2 ± 15.4	*0.237[(a)]*
Body mass index, mean ± S.D.	26.0 ± 5.6	26.4 ± 6.6	25.3 ± 5.3	*0.547[(a)]*
Comorbidities, n (%)				
SAH	26 (60.4)	8 (44.4)	18 (72.0)	*0.055 [(b)]*
SAH + DM	6 (14.0)	2 (11.1)	4 (16.0)	
SAH + CHF	11 (25.6)	8 (44.4)	3 (12.0)	
Smoking, n (%)				
Yes	9 (20.9)	2 (11.1)	7 (28.0)	*0.124 [(b)]*
No	34 (79.1)	16 (88.9)	18 (72.0)	
Alcohol intake, n (%)				
Yes	(0.0)	0 (0.0)	0 (0.0)	*1.00[(b)]*
No	43 (100.0)	18 (100.0)	25 (100.0)	
Physical exercise, n (%)				
Yes	(0.0)	0 (0.0)	0 (0.0)	*1.00[(b)]*
No	43 (100.0)	18 (100.0)	25 (100.0)	
Family history of kidney disease, n (%)				
Yes	9 (20.9)	3 (16.7)	6 (24.0)	*0.711[(b)]*
No	34 (79.1)	15 (83.3)	19 (76.0)	
Time in hemodialysis (years), mean ± S.D.				
	4.4 ± 2.6	4.5 ± 2.4	4.2 ± 2.7	*0.704[(a)]*
Kt/V				
< 1.2	15 (34.9)	7 (38.9)	8 (32.0)	*0.640[(b)]*
≥ 1.2	28 (65.1)	11 (61.1)	17 (68.0)	
GOA, n (%)				
Appropriate	2 (4.6)	2 (11.1)	0 (0.0)	*0.169[(b)]*
Mild/Moderate	41 (95.4)	16 (88.9)	25 (100.0)	

Source: Elaborated from the author (2023).

Subtitle: 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≤0.05).

When the patients were stratified according to AGE serum levels, almost all general characteristics of the sample investigated was similar comparing patients with low and high glycation results. However, inadequate dialysis dose (KT/V<1.2) was mainly identified in patients with increased AGE levels compared to patients with reduced AGE accumulation (P<0.05). Nutritional status remained unchanged after AGE stratification (P>0.05) (TABLE 2).

Table 2 – General characteristics of all hemodialysis patients stratified according to low (<) and high (>) advanced glycosylation end products (AGE) serum levels

Variables	AGE < 14.6 (n= 21)	AGE > 14.6 (n = 22)	*P value*
Age (years), mean ± S.D.	54.5 ± 16.6	52.5 ± 19.5	*0.721* [a]
Body mass (kg), mean ± S.D.	70.7 ± 13.1	66.0 ± 14.1	*0.266*[a]
Body mass index, mean ± S.D.	26.2 ± 5.6	23.9 ± 4.9	*0.158*[a]
Comorbidities, n (%)			
SAH	12 (57.1)	15 (68.2)	
SAH + DM	4 (19.1)	2 (9.1)	*0.613*[b]
SAH + CHF	5 (23.8)	5 (22.7)	
Smoking, n (%)			
Yes	4 (19.1)	5 (22.7)	
No	17 (80.9)	17 (77.3)	*1.00*[b]
Alcohol intake, n (%)			
Yes	0 (0.0)	0 (0.0)	*1.00*[b]
No	21 (100.0)	22 (100.0)	
Sedentary, n (%)			
Yes	16 (100.0)	28 (100.0)	*1.00*[b]
No	0 (0.0)	0 (0.0)	
Family history of KD, n (%)			
Yes	3 (14.3)	6 (27.3)	*0.465*[b]
No	18 (85.7)	16 (72.7)	
Time HD (years), mean ± S.D.	3.33 ± 1.31	4.1 ± 3.5	*0.358*[a]
Kt/V, mean ± S.D.	1.5 ± 0.3	1.2 ± 0.2	***<0.001***[a]
Kt/V			
< 1.2	4 (19.1)	15 (34.9)	***0.001***[b]
≥ 1.2	17 (80.9)	7 (61.1)	
Global objective assessment, n (%)			
Appropriate nutrition	2 (9.5)	0 (0.0)	*0.232* [b]
Mild/moderate malnutrition	19 (90.5)	22 (100.0)	

Source: Elaborated from the author (2023).

Subtitle: KD, kidney disease; HD, hemodialysis, DM, diabetes mellitus; SAH, systemic arterial hypertension; CHF, congestive heart failure; GOA: global objective assessment. AGE, advanced glycosylation end products. P values: (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 AGE groups (P≤0.05).

As indicated in Table 3, the groups stratified and according to AGE circulating levels exhibited marked and expected differences in this parameter (P<0.05). Patients with higher AGE accumulation also presents increased was increased urea (before and after HD) serum levels and white blood cell count compared with patients in the lower AGE stratum (P<0.05). Creatinine, blood glucose, albumin, hematocrit, iron, ferritin, and TSI levels were similar in patients categorized in both AGE stratum (P>0.05).

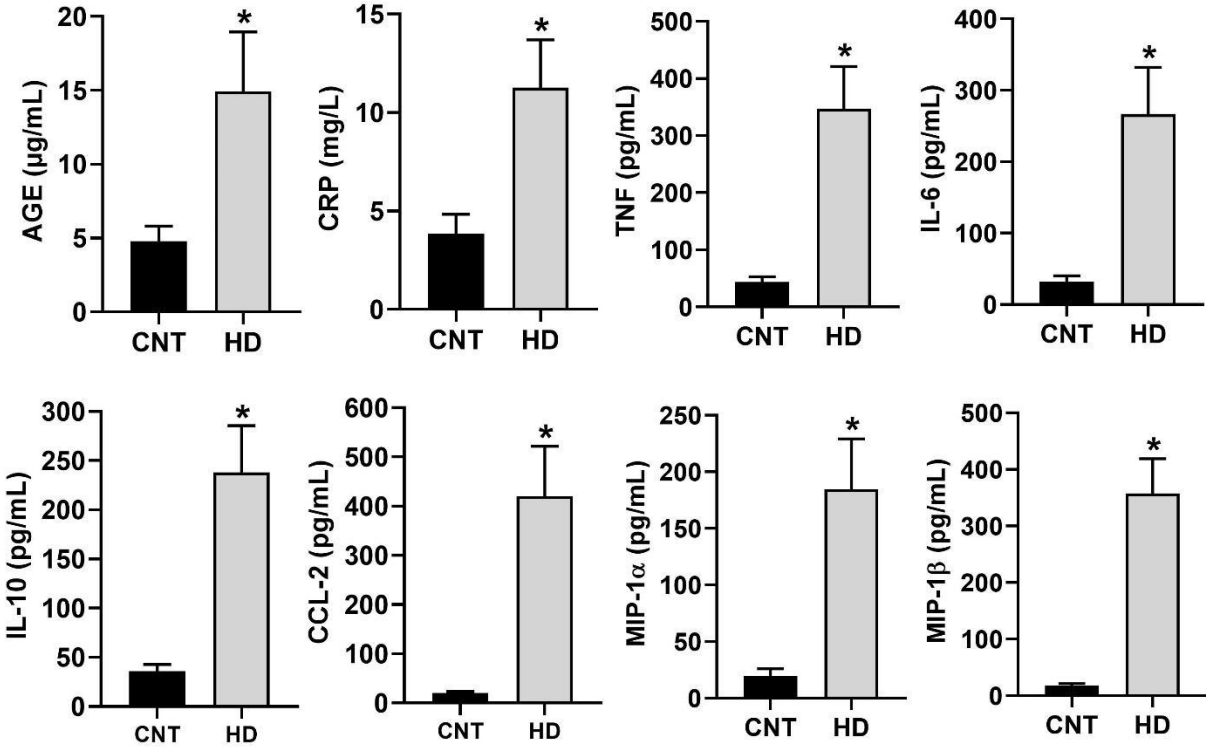
Table 3 - Blood and serum biochemical parameters of hemodialysis (HD) patients stratified according to low (<) and high (>) advanced glycosylation end products (AGE) serum levels

Variables	AGE < 14.6 (n= 21)	AGE > 14.6 (n = 22)	*P value**
AGE (µg/mL)	11.61 ± 2.00	18.28 ± 2.39	***<0.001***
Pre-HD urea (mg/dL)	100.15 ± 30.08	126.17 ± 36.44	***0.035***
Post-HD urea (mg/dL)	35.77 ± 11.21	49.01 ± 20.27	***0.010***
Creatinine (mg/dL)	12.61 ± 3.33	15.68 ± 7.25	*0.089*
Leucocytes × 10^2/µl	61.04 ± 12.55	80.11 ± 16.39	***<0.001***
Blood glucose (mg/dL)	145.15 ± 65.23	147.11 ± 77.39	*0.929*
Total Protein (mg/dL)	3.98 ± 0.41	3.73 ± 0.55	*0.102*
Albumin (g/dL)	270.01 ± 212.48	268.25 ± 209.15	*0.978*
Hematocrit (%)	32.51 ± 4.19	33.96 ± 6.41	*0.393*
Hemoglobin (g/dL)	11.26 ± 1.59	10.88 ± 1.95	*0.491*
Iron (mg/dL)	17.55 ± 6.13	17.92 ± 8.04	*0.867*
Ferritin (ng/dL)	6.98 ± 0.91	7.11 ± 0.74	*0.608*
TSI (%)	20.13 ± 9.28	21.69 ± 10.05	*0.601*

Source: Elaborated from the author (2023).
Subtitle: AGE: advanced glycation end products, TSI: transferrin saturation index. *P values in bold indicates significant difference (P≤0.05) among the groups stratified by AGE levels and obtained from Student's t test or Mann-Whitney U test, according data distribution.

As represented in Figure 1, health control volunteers exhibited lower AGE, CRP, TNF, IL-6, IL-10, MCP-1, MIP-1α and MIP-1β serum levels. All these parameters were increased in HD patients (P<0.05), corroborating the systemic pro-inflammatory status associated with ERSD.

Figure 1 – Advanced glycation end products (AGE), c reactive protein (CRP), cytokines and chemokines serum levels in health control volunteers (CNT) and hemodialysis (HD) patients



Source: Elaborated from the author (2023).
Subtitle: * The symbol indicates significant difference (P≤0.05) among the groups from Student's t test or Mann-Whitney U test, according data distribution.

As indicated in Table 4, the groups stratified and according to AGE circulating levels exhibited similar CRP and MIP-1α levels (P>0.05). Conversely, this stratification revealed marked differences in the other cytokines and chemokines investigated. Thus, patients with higher AGE accumulation also presents increased TNF, IL-6, IL10, CCL-2 and MIP-1β circulating levels (P>0.05).

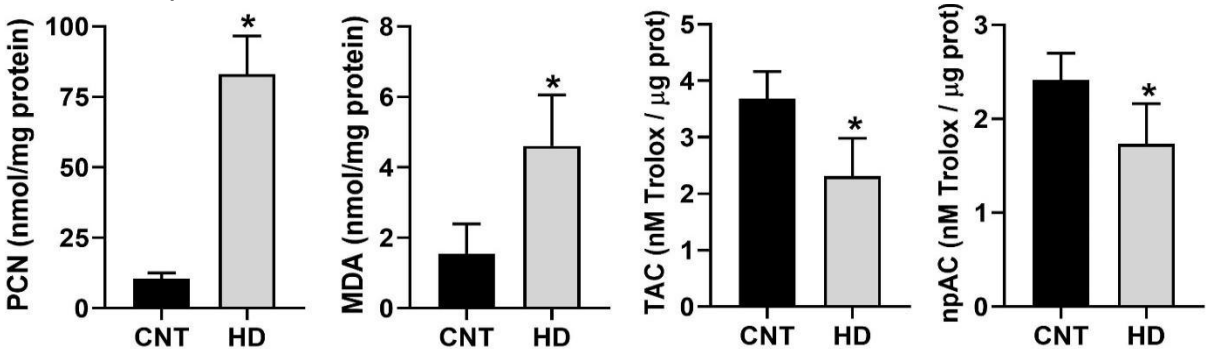
Table 4 - Comparison between cytokines, chemokines, and c reactive protein (CRP) serum levels in hemodialysis patients stratified according to low (<) and high (>) advanced glycosylation end products (AGE) serum levels

Variables	AGE < 14.6 (n= 21)	AGE > 14.6 (n = 22)	*P value*
General inflammatory marker			
C reactive protein (mg/L)	9.36 ± 1.84	13.14 ± 2.01	*0.695*
Cytokines (pg/mL)			
TNF	279.96 ± 34.69	414.94 ± 18.03	***<0.001***
IL-6	209.62 ± 37.21	323.76 ± 24.21	***<0.001***
IL-10	203.51 ± 8.95	272.04 ± 61.01	***<0.001***
Chemokines (pg/mL)			
CCL-2	334.33 ± 50.61	506.04 ± 54.55	***<0.001***
MIP-1α	169.65 ± 36.22	199.12 ± 60.46	*0.232*
MIP-1β	322.72 ± 56.20	408.59 ± 44.17	***<0.001***

Source: Elaborated from the author (2023).
Subtitle: *P values in bold indicates significant difference (P≤0.05) among the groups stratified by AGE levels and obtained from Student's t test or Mann-Whitney U test, according data distribution.

As indicated in Figure 2, health control volunteers exhibited lower PCN and MDA serum levels. Conversely, TAC and npAC were increased in health volunteers. While PCN and MDA levels were increased (P<0.05), TAC and npAC were reduced in HD patients (P<0.05), corroborating the systemic pro-oxidant status associated with ERSD.

Figure 2 – Protein carbonyl (PCN), malondialdehyde (MDA), total antioxidant capacity (TAC), and non-protein antioxidant capacity (npAC) in serum samples of health control volunteers (CNT) and hemodialysis (HD) patients



Source: Elaborated from the author (2023).
Subtitle: * The symbol indicates significant difference (P<0.05) among the groups from Student's t test or Mann-Whitney U test.

As shown in Table 5, the groups stratified and according to AGE circulating levels exhibited marked differences in pro-oxidant and antioxidant markers. Thus, patients with higher AGE accumulation exhibited increased PCN and MDA levels (P<0.05), as well as reduced TAC and npAC in levels (P<0.05) in serum samples compared to patients in the lower AGE stratum.

Table 5 - Comparison between serum levels of oxidant and antioxidant markers in hemodialysis patients stratified according to low (<) and high (>) advanced glycosylation end products (AGE) serum levels

Variables	AGE < 14.6 (n= 21)	AGE > 14.6 (n = 22)	*P value*
PCN (nmol/mg protein)	72.33 ± 5.65	93.82 ± 10.18	***<0.001***
MDA (nmol/mg protein)	3.54 ± 0.74	5.68 ± 1.16	***<0.001***
TAC (nM Trolox / µg protein)	1.60 ± 0.29	2.62 ± 0.37	***<0.001***
npAC (nM Trolox / µg protein)	1.39 ± 0.19	2.08 ± 0.29	***<0.001***

Source: Elaborated from the author (2023).
Subtitle: PCN, protein carbonyl. MDA, malondialdehyde. TAC, total antioxidant capacity. npAC, non-protein antioxidant capacity. *P values in bold indicates significant difference (P≤0.05) among the groups stratified by AGE levels and obtained from Student's t test or Mann-Whitney U test.

As reported in Table 6, CRP, TNF, IL-6, and CCL-2 exhibited positive and significant correlation with AGE serum levels in both groups stratified according to this glycosylation parameter (P<0.05). MIP-1α and MIP-1β were positively correlated and IL-10 was negatively correlated with AGE only in patients with higher AGE accumulation (P<0.05).

Table 6 - Correlation between low (<) and high (>) advanced glycosylation end products (AGE), cytokine and chemokine serum levels hemodialysis patients

Variables	AGE < 14.6 (n= 21)		AGE > 14.6 (n = 22)	
	Coefficient (R)	*P value*	Coefficient (R)	*P value**
General inflammatory marker				
C reactive protein (mg/L)	0.655	***0.001***	0.801	***<0.001***
Cytokines				
TNF (pg/mL)	0.697	***<0.001***	*0.829*	***<0.001***
IL-6 (pg/mL)	0.662	***0.001***	0.798	***<0.001***
IL-10 (pg/mL)	-0.409	*0.065*	-0.783	***<0.001***
Chemokines				
CCL-2 (pg/mL)	0.558	***0.008***	0.878	***<0.001***
MIP-1α (pg/mL)	0.414	*0.062*	0.469	***<0.032***
MIP-1β (pg/mL)	0.322	*0.155*	0.891	***<0.001***

Source: Elaborated from the author (2023).
Subtitle: *P values in bold indicate significant correlation (Pearson correlation test) of AGE with C reactive protein, cytokine and chemokines serum levels ($P \leq 0.05$).

The results of linear regression for CRP, cytokines and chemokines as dependent variables are shown in Table 7. Corroborating the correlation findings, the regression models indicated that CPR, TNF, IL-6 and CCL-2 variability was partially explained by AGE levels in both groups with low and high AGE stratum ($P<0.05$). In addition, AGE serum levels presented some significance for predicting IL-10, MIP-1α and MIP-1β only in patients with higher AGE accumulation ($P<0.05$).

Table 7 - Linear regression model with c reactive protein, cytokines and chemokines as dependent variables according low (<) and high (>) advanced glycosylation end products (AGE) serum levels

Variables	AGE < 14.6 (n= 21)			AGE > 14.6 (n = 22)		
	β	R^2	*P value*	*β*	R^2	*P value*
Inflammatory effectors						
C reactive protein (mg/L)	3.646	0.429	***0.001***	1.482	0.642	***<0.001***
Cytokines						
TNF *(pg/mL)*	0.08284	0.485	***<0.001***	0.1597	0.686	***<0.001***
IL-6 *(pg/mL)*	0.08132	0.4381	***0.001***	0.1236	0.636	***<0.001***
IL-10 *(pg/mL)*	-0.5469	0.167	*0.065*	-0.04998	0.612	***<0.001***
Chemokines						
CCL-2 *(pg/mL)*	0.06856	0.311	***<0.008***	0.04984	0.770	***<0.001***
MIP-1α *(pg/mL)*	0.1337	0.171	*0.062*	0.08426	0.219	***0.032***
MIP-1β *(pg/mL)*	0.1108	0.103	*0.155*	0.06062	0.794	***<0.001***

Source: Elaborated from the author (2023).
Subtitle: P values in bold indicate statistical significance for each predictor in the regression model (P≤0.05).

As indicated in Table 8, PCN and MDA exhibited positive and significant correlation with AGE serum levels in both groups stratified according to this glycosylation parameter (P<0.05). Conversely, TAC and npAC were inversely correlated with AGE only in patients with higher AGE accumulation (P<0.05).

Table 8 - Correlation between low (<) and high (>) advanced glycosylation end products (AGE), and serum levels oxidant and antioxidant markers in hemodialysis patients

Variables	AGE < 14.6 (n= 21)		AGE > 14.6 (n = 22)	
	Coefficient (R)	*P value*	Coefficient (R)	*P value**
Oxidant markers				
PCN (nmol/mg protein)	0.544	***0.010***	0.819	***<0.001***
MDA (nmol/mg protein)	0.527	***0.014***	*0.810*	***<0.001***
Antioxidant markers				
TAC (nM Trolox / µg protein)	-0.378	*0.091*	-0.805	***<0.001***
npAC (nM Trolox / µg protein)	-0.367	*<0.101*	-0.680	***<0.001***

Source: Elaborated from the author (2023).
Subtitle: PCN, protein carbonyl. MDA, malondialdehyde. TAC, total antioxidant capacity. npAC, non-protein antioxidant capacity. *P values in bold indicate significant correlation (Pearson correlation test) of AGE with C reactive protein, cytokine and chemokines serum levels ($P \leq 0.05$).

The results of linear regression for pro-oxidant and antioxidant markers as dependent variables are shown in Table 9. In line with the correlation results, the regression models indicated that PCN and MDA variability was partially explained by AGE levels in both groups with low and high AGE stratum (P<0.05). In addition, AGE serum levels presented predictive significance for TAC and npAC in patients with higher AGE accumulation (P<0.05).

Table 9 - Linear regression model with protein carbonyl, malondialdehyde and antioxidant capacity as dependent variables according low (<) and high (>) advanced glycosylation end products (AGE) serum levels

Variables	AGE < 14.6 (n= 21)			AGE > 14.6 (n = 22)		
	β	R^2	*P value*	*β*	R^2	*P value*
Oxidant markers						
PCN (nmol/mg protein)	0.6515	0.295	***0.010***	10.35	0.670	***<0.001***
MDA (nmol/mg protein)	5.153	0.277	***0.014***	2.532	0.655	***<0.001***
Antioxidant markers						
TAC (nM Trolox / µg protein)	-18.20	0.091	*0.084*	-7.607	0.647	***<0.001***
npAC (nM Trolox / µg protein)	-28.08	0.135	*0.101*	-12.12	0.462	***<0.001***

Source: Elaborated from the author (2023).

Subtitle: PCN, protein carbonyl. MDA, malondialdehyde. TAC, total antioxidant capacity. npAC, non-protein antioxidant capacity. P values in bold indicate statistical significance for each predictor in the regression model (P≤0.05).

4 DISCUSSION

The present study investigated the relevance of advanced glycation end products as predictors of inflammation and redox imbalance in ESRD patients undergoing HD. From an integrated context, we confirmed that HD patients manifested a dramatic systemic proinflammatory and prooxidant status, with unequivocal cytokines and oxidative markers upregulation and antioxidant defenses down-regulation compared to their healthy control peers. As expected, our case series presented nutritional characteristics, comorbidities and dialysis dose consistent with clinimetric delimiters classically established for this population (Cobo et al., 2016; Colman et al., 2005; Miskulin et al., 2009). Accordingly, a marked frequency of sedentary lifestyle, overweight, unbalanced nutritional status and comorbidities such as diabetes mellitus and SAH seems to be more a rule than an exception in HD patients (Miskulin et al., 2009; Silva et al., 2019). Unfortunately, patients exposed to a limited period in HD (e.g., 5-7 years) was also identified, an expected finding considering that annual mortality is around 9%, with 40-50% 5-year survival (Nordio et al., 2012). Given these characteristics, there is no doubt about the need for multi-professional assessments and effective therapeutic interventions, since controlling nutritional status and comorbidities associated with ESRD pathogenesis is essential to attenuate clinical decay, improving HD quality and patient survival (Cupisti et al., 2018; Silva et al., 2019; Pei et al., 2019). However, ESRD management is a unique therapeutic challenge, since medical and nutritional interventions currently available are not yet capable of neutralizing all pathological outcomes associated with uremic toxicity (De Rosa et al., 2017; Faria and Pinho, 2021).

From patients' stratification using a median-based AGE cut-off index, only dialysis dose (Kt/V) was markedly reduced, while leucocytes distribution, pre- and post-HD urea levels were increased in patients with higher AGE accumulation. This is an interesting finding, especially considering that uremic toxins retention, including urea, may be directly related to HD efficiency (Castillo-Rodríguez et al., 2017; Vanholder et al., 2018). In this case, it is indeed expected that patients exposed to lower clearance of uremic toxins are more likely to manifest inflammatory and redox imbalance (Cobo et al., 2018; Pieniazek et al., 2021). Alone, leukocytes distribution does not represent unequivocal evidence of systemic inflammation. Thus, regardless

of this cellular indicator, the set of patients investigated demonstrated disproportionately high cytokine circulating levels compared to their healthy control peers. In addition, patients with higher AGE retention exhibited higher levels of all investigated cytokines compared to patients below the AGE cut-off, except for MIP-1α. Accordingly, leukocytes distribution may be related to systemic inflammation in these patients, a hematological condition whose etiopathogenesis is complex, multifactorial (e.g.; uremic toxicity, type of dialysis membrane, vascular access, biofilm formation on venous catheters, dialysis solutions, immunological deviation associated with comorbidities) and still little understood (Perkins et al., 2021; Prasad et al., 2016).

In addition to the increased production, difficult to remove and potent immunobiological reactivity reinforce the cytokines role as uremic toxins (Castillo-Rodríguez et al., 2017; Wolley and Hutchison, 2018). Accordingly, greater accumulation of these molecules has been consistently associated with increased risk for CVD development (Lim et al., 2021; Sasaki et al., 2021), as well as deaths from cardiovascular events (Dai et al., 2017; Sasaki et al., 2021) and general causes (Msaad et al., 2019; Wang et al., 2017) in ERSD patients. These molecules orchestrate the systemic inflammatory syndrome (Mihai et al., 2018; Wang et al., 2022), which is closely correlated to the activation of prooxidant mechanisms involved in the pathogenesis of cardiovascular complications almost invariably detected in HD patients (Pieniazek et al., 2021; Sasaki et al., 2021). In fact, the investigated patients exhibited worrying levels of oxidized proteins and lipids compared to their healthy control peers, reinforcing the evidence of oxidative metabolic overload (Ebert et al., 2021; (Pieniazek et al., 2021). Accordingly, this process was potentially exacerbated by the down-regulation of protein and non-protein antioxidant defenses, which are often impaired in these patients (Pieniazek et al., 2021; Poulianiti et al., 2016). This antioxidant fragility is not completely understood, but it is potentially associated with catabolic condition coupled with nutritional imbalance and depletion of antioxidant effectors in response to chronic uremic-oxidative stress (Epifânio et al., 2018; Slee 2012). Oxidative stress is not a trivial manifestation in ESRD patients. In addition to molecular oxidation integrating CVD pathogenesis in this population (Daenen et al., 2019; Podkowińska and Formanowicz 2020), increased circulating levels of oxidized proteins and lipids, as well as the depletion of antioxidant effectors have been highlighted as predictors

of cardiovascular deterioration and mortality in HD patients (Irazabal and Torres 2020; Ratliff et al., 2016). Interestingly, oxidative status has also been associated with dialysis dose/adequacy, as indicated by Kt/V (Ignace et al., 2009; Stepanova et al., 2019). Thus, reduced lipid and proteins oxidation does not appear to necessarily result from the direct elimination of these oxidative overload markers, being mainly related to a more effective clearance of uremic toxins and attenuation of systemic inflammation, since they act in coupled processes (Cohen 2020; Irazabal and Torres 2020). In this sense, is desirable that oxidative stress be continuously monitored and corrected during HD (Locatelli and Canaud et al., 2012).

Interestingly, oxidative stress markers (increased MDA and PCN *vs.* reduced TAC and npAC levels) followed the same trend as cytokines, indicating that greater AGE retention establishes a notorious interface between systemic inflammation and oxidative stress in ESRD patients. Although this relationship has been suggested in previous studies (Molinari et al., 2021; Stinghen et al., 2016), the predictive relevance of AGE for estimating proinflammatory and prooxidant status in ESRD patients undergoing HD is still overlooked. Accordingly, we identified that AGE accumulation was directly correlated with circulating levels of inflammatory (CRP, TNF, IL-6, CCL-2) and prooxidant markers (MDA and PCN); independent of the AGE cut-off index. However, negative correlation of AGE with IL-10, TAC and npAC, as well as positive correlation with MIP-1α and MIP-1β occurred only in patients above the AGE cut-off. These findings indicate potential variability in AGE relationship with cytokines and oxidative markers (pro-oxidants *vs.* antioxidants) expressed by HD patients, a poorly understood phenomenon that requires further mechanistic studies. These relationships also indicated AGE-specific relevance in partially predicting cytokines, oxidant and antioxidant variability in HD patients. Overall, this predictive value was quite limited for cytokines (< 49%) and oxidant markers (< 30%) for patients below the AGE cut-off. However, the variability of these molecules was better estimated by AGE levels in patients with greater AGE retention. Thus, it is not possible to rule out a more intricate bidirectional causal relationship between AGE accumulation, cytokines production and oxidative stress in patients exposed to greater uremic overload (Cohen 2020; Molinari et al., 2021).

The relevance of this study is essentially based on the urgent need to improve inflammatory and oxidative status monitoring of patients on maintenance HD, admitting that these processes modulate the pathological outcomes attributed to

ESRD (Ebert et al., 2021; Wang et al., 2017). Undeniably, early mortality is the greatest risk imposed on these patients, which is 10-30 times greater compared to the general population (Lee and Son, 2021) and can manifested in about 63.4% cases after 5 years and 76% after 8 years of HD initiation (de Arriba et al., 2021). Accordingly, we investigated molecules directly involved in the etiopathogenesis of cardiovascular complications, the leading causes of death in HD patients (Dai et al., 2017; Lim et al., 2021). Particularly, cytokines such as IL-6 and TNF are almost invariably upregulated (4- to 5-fold increase) in HD patients compared to health controls. IL-6 and TNF are potent immunological effectors of the chronic inflammatory overload in ESRD (Castillo-Rodríguez et al., 2017), playing a direct role in pro-oxidant and thromboembolic events, leucocytes recruitment and activation, and atherosclerosis development (Castillo-Rodríguez et al., 2017; Hartman and Frishman, 2014; Wanner et al., 2016). These proinflammatory cytokines have also been found to be relevant predictors of CVD and risk of death in HD patients (Dai et al., 2017; Wang et al., 2017). Conversely, IL-10 counterregulates IL-6 and TNF effects, exhibiting protective anti-inflammatory properties in ESRD (Castillo-Rodríguez et al., 2017). However, the cardioprotective effects attributed to IL-10 are limited, as this cytokine is often unable to counteract systemic toxicity and high-grade inflammation (Chen et al., 2021; Stenvinkel et al., 2005), evidencing the role of uremic overload in the pathophysiology of the oxidative-inflammatory syndrome in HD patients (Jiang et al., 2021; Perkins et al., 2021). Interestingly, reduced IL-10 production was previously associated with increased cardiovascular mortality in HD patients (Girndt et al., 2002), reinforcing the relevance of this cytokine as a marker of pro- and anti-inflammatory immune balance, and as a prognostic indicator in these patients.

Circulating chemokines have also been consistently used as markers of systemic inflammation in HD patients (De Oliveira Junior et al., 2020). Interestingly, the correlation between AGE with CCL-2, MIP-1α and MIP-1β in patients with higher AGE retention indicated that AGE may exert a predictive value when systemic inflammation and oxidative stress are more pronounced. Molecules such as CCL-2, MIP-1α, and MIP-1β are centrally implicated in monocytes/macrophages recruitment, as well as renal and CVD pathogenesis, including atherosclerosis in chronic kidney disease (CKD) patients (Okumoto et al., 2009; Chung and Lan, 2011; de Oliveira Junior et al., 2020). Accordingly, CCL-2 upregulation was also identified as a

promising immunological biomarker for prognosis and/or as a therapeutic target in CKD, although the reference values for this chemokine still need to be established (Schettini et al., 2022). During atherogenesis, CCL3, and CCL4 also exhibits prooxidant and procoagulant effects, aggravating cardiovascular risk by stimulating a prothrombotic state in HD patients (Pawlak et al., 2006). Unfortunately, predictive MIP1-α and MIP1-β markers, as well as the diagnostic and prognostic relevance of these molecules in HD are poorly explored. Thus, the predictive potential of AGE deserves to be further explored, as there may be a critical threshold for AGE retention and for inflammation severity above which these chemokines may be clinically relevant to guide HD patient management.

In line with the prognostic relevance of cytokines and chemokines, oxidative stress markers such reactive oxygen metabolites (Sasaki et al., 2021), MDA and PCN are also indicated as predictors of mortality by cardiovascular events (Malekmakan et al., 2020) and all-cause mortality in HD patients (Song et al., 2020; Zuo et al., 2022). In fact, CVD diagnosis is often accompanied by marked ROS production and molecular oxidation (lipids, proteins and DNA) in ESRD (Podkowińska and Formanowicz 2020). This condition is also closely correlated with deterioration of enzymatic and non-enzymatic antioxidant defenses in HD patients, which seems to be associated with HD-induced antioxidant substance losses and molecular exhaustion determined by continuous metabolism of radical and non-radical reactive species (Canaud et al., 1999; Stępniewska et al., 2015). In addition to the evident metabolic risk provided by prooxidant metabolites accumulation, and its negative impact on patient management and clinical evolution, oxidative stress needs to be adequately monitored and corrected as part of dialysis therapy itself. Thus, identifying predictors of inflammatory and redox status, such as circulating AGE levels, can be a valuable opportunity to improve the evaluation of efficacy and seek optimized interventions to obtain the best clinical prognosis for HD patient, such as adjustment of hemodialysis hemocompatibility and antioxidant supplementation (Canaud et al., 1999).

5 CONCLUSION

Taken together, our findings indicated that HD patients exhibit a hyperinflammatory state and a redox imbalance associated with uremic-oxidative toxicity. These manifestations are associated with variable accumulation of AGE, which is relevant to predict the systemic inflammatory and oxidative state. Apparently, the predictive relevance of AGEs for cytokines, chemokines, pro-oxidant markers and antioxidants is greater in patients with intense AGE retention. Thus, the stratification of patients guided by circulating levels of AGE may be relevant to track and monitor patients on maintenance HD according to exposure to increased systemic inflammatory and oxidative overload, conditions known to be associated with worse prognosis and higher risk of mortality in this condition. population. In this sense, identifying robust predictors and establishing reference values for immunological and oxidative markers represents a new challenge with the potential to reorient the follow-up and treatment of patients on HD. It is suggested that further studies be carried out to deepen improvements in the follow-up of these patients.

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