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Conversion from Hemodialysis to Hemodiafiltration Affects the Innate Immunity of Individuals with Chronic Kidney Disease

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ABSTRACT

Background: Once a diagnosis of chronic kidney disease (CKD) stage 5 is confirmed, possible treatments include kidney replacement therapies, such as hemodialysis (HD) and hemodiafiltration (HDF). HD removes low-molecular-weight molecules, while HDF purges small and large molecules, favoring the reduction of oxidative stress.
Objectives: This study evaluated the hematological, biochemical and immunological parameters of individuals with CKD treated with HD who later converted to HDF.
Methods: This is a descriptive, retrospective and comparative study carried out with 25 individuals whose HD treatment was later converted to HDF (convenience sample).
Results: Data were analyzed in blood samples (cells and serum). Patient`s etiologies were type II Diabetes Mellitus (DM) (48%) and Systemic Arterial Hypertension (SAH) (32%). HDF reduced serum levels of erythropoietin (EPO), glucose, aspartate aminotransferase, and β2-microglobulin and the EPO resistance index and increased levels of alkaline phosphatase and C-reactive protein. HDF normalized the phagocytic index with 5 or 20 yeasts/cell and normalized the stimulated corpuscular index but increased TNF and IL-4 production compared to HD. Furthermore, HDF normalized the basal production of O_2 and its production in the absence of phagocytosis, but when compared to HD, HDF increased the production of O_2 in the presence or absence of yeast ingestion.
Conclusion: Our results indicate that HDF is efficient in treating patients with CKD by improving most of immunological markers and maintaining the treatment efficacy. Considering that HDF is rarely used in Brazil, this a study is suggested to promote greater visibility and acceptance of HDF among patients and the medical community, aiming at its future implementation in the public health system.

Keywords: Blood cells; Biomarkers; Hemodiafiltration; Hemodialysis; Chronic kidney disease

INTRODUCTION

Chronic Kidney Disease (CKD) is commonly characterized by a low glomerular filtration rate (<60 mL/min/1.73 m²) associated with proteinuria, tissue damage in the kidneys and time of clinical manifestation [1]. The National Kidney Foundation defined CKD as an abnormality of kidney structure or function regardless of cause or specific clinical presentation and proposed a staging system based on the level of glomerular filtration rate (Stages I to V) [2]. CKD has high mortality rates, making it a challenge for public and private health systems worldwide [3]. In Brazil, it is estimated that 1.42% of the population has CKD [4].

Among the available kidney replacement therapies (KRTs), hemodialysis (HD) is widespread in Brazil, while hemodiafiltration

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(HDF) is more used in the US, Europe and Japan [5,6]. While HD purifies low molecular weight molecules, toxins and excess water from the blood through a dialyzer [7]. HDF purges small and large molecules by combining diffusion and convection in the same high-flow device [5,6]. Moreover, HDF provides larger convective volumes of ultrafiltration flow [8].

Different studies have reported the benefits of HDF for people with CKD. Among these, improvement of hematological and biochemical parameters, reduction of oxidative stress and reduction of hospitalization and mortality rate stand out [7-12]. Thus, considering all reported benefits, this study aimed to evaluate the hematological, biochemical and immunological data of individuals with CKD treated with HD who later converted to HDF. As Brazilian public health system does not apply HDF treatment, our study may provide solid data to support institutional decision of adopting alternative treatments.

METHODS

Study design, individuals, and inclusion and exclusion criteria. This is a descriptive, comparative, experimental, cross-sectional, paired study to evaluate the effects of HDF on hematological, biochemical and immunological data of individuals with CKD (12 women and 13 men) undergoing treatment for more than 6 months in the Nephron Clinic in Brasilia, Federal District, Brazil. A control group (n=7) was entered into the study to determine baseline levels of *in vitro* immunological parameters.

The study included adults and elderly individuals (66 ± 10 years) with stage V CKD, definitive vascular access and blood pump flow (BPF) ≥ 400 mL/min. Individuals who did not agree to participate in the study were excluded from the study, as were those with temporary vascular access or BPF ≤ 399 mL/min.

HD and HDF Parameters

FX60 high-flow dialyzers and 4008S machines (Fresenius Medical Care, Bad Homburg, Germany) for HD and HDF were used for this study. The parameters consisted of ultrapure water (0.1 CFU/mL) and a replacement volume of 100 mL/min, and the dialysate was composed of sodium (138 to 140 mg/dL), potassium (1.5 to 2.0 mg/dL), calcium (2.5 to 3.0 mg/dL), bicarbonate (44 mg/dL) and glucose (100 mg/dL).

Blood Sampling, Hematological and Biochemical Evaluation

To perform hematological and biochemical assays, 20 mL of peripheral blood was collected from each patient (n=25) at two time points:

1. before treatment with HD and
2. 12 weeks after conversion to HDF.

All hematological and biochemical analyses were performed at Sabin Medicina Diagnostica. Hematological parameters for erythrocytes and leukocytes were analyzed by fluorescent flow cytometry and impedance (XN10TM Sysmex Inc., Mississauga, Ontario, Canada). Biochemical analyses were performed on a Cobas 8000 modular analyzer (Roche Diagnostics, California, USA). Total cholesterol was analyzed by the esterase/oxidase method, urea by the urease method with glutamate dehydrogenase, creatinine by the amidinohydrolase/oxidase method, albumin by the bromocresol green method and parathyroid hormone by electro chemiluminescence. Normal reference values were applied for hematological and biochemical data.

Assessment of cell function by cytological assays for the cytological assays, leukocytes were obtained on histological slides previously marked (diameter=1 cm) for cell adhesion. Peripheral blood aliquots obtained from CKD patients (n=25) and control patients (n=7) were added in duplicate to Hanks-Triz medium and subsequently incubated in a humid chamber at 37°C for cell adhesion to assess phagocytic capacity (40 µL; 60 minutes), superoxide production O_2 (40 µL; 45 minutes) and lipid corpuscles (75 µL; 120 minutes). To remove erythrocytes and non-adhered cells, the wells were washed with a PBS solution at 37°C. A single observer evaluating 200 cells using optical microscopy (1000 x) performed all analyses.

Phagocytic index: Cells were incubated (30 minutes) with 40 µL of a suspension of *Saccharomyces cerevisiae* (5 or 20 yeasts/cell) previously sensitized with 10% of the individual's own serum (phagocytosis by opsonins) or with fetal bovine serum (phagocytosis by molecular standards). Then, yeasts that remained on the suspension medium were removed, and the cells were dried with hot air, fixed with methanol (1 min) and stained with 10% Giemsa solution for 10 minutes. The results were expressed by the phagocytic index (IF) (average of ingested yeasts x percentage of cells involved in phagocytosis).

Lipid bodies: After adherence, the cells were incubated (30 minutes) with yeast suspension (5 lev/cell) previously sensitized with 10% of the individual's own serum. After removal of non-ingested yeasts and fixation with 4% paraformaldehyde (15 minutes), the cells were washed with a sequence of solutions (2x with PBS; 1x with 0.5 mL of 60% isopropyl alcohol; 2x with distilled water), stained with Oil Red followed by Harris Hematoxylin, and protected with coverslips in gelatinous medium. The results were expressed by the corpuscular index (CI), which is the product of the average of lipid corpuscles and the percentage of cells with corpuscles.

Superoxide radical production (O_2): After adherence, cells (monocytes, neutrophils and eosinophils) were incubated (20 minutes) with nitro blue tetrazolium (NBT) solution (20 µL) in Hanks-Triz medium (basal) and added to a suspension of 20 yeasts/cell to stimulate O_2 production. This method is based on the reduction of NBT by cells in the presence of O_2 (yellow salt to blue pigment in the cytoplasm). Cells were fixed (methanol) and stained with a safranin solution (2.5 g) for 5 minutes. The results were expressed as the percentages of cells that presented the following:

1. Blue pigment+yeasts;
2. Blue pigment without yeasts;
3. Without blue pigment+yeasts; and
4. Without blue pigment or yeasts.

Cytokine Quantification

To perform cytokine assays, plasma patients were previously stored at -80°C before use. CBA single plex flex set and Cell

Signaling Master Buffer (BD™ CBA, USA) were used according to manufacturer instructions. The capture beads samples were read at flow cytometer LSRFortessa™ (BD™, USA) according to the manufacturer instructions. Data was converted to FCAP Array™ Software Version 3.0 (BD™, USA) and the results were presented in pg/mL.

Statistical Analysis

Statistical analysis of data was performed by Kolmogorov-Smirnov and Bartlett tests in order to check normality and variability parameters. For multiple data comparisons ANOVA or Kruskal-Wallis were performed to parametric or non-parametric data respectively. T-Student or Mann-Whitney tests were performed when two independent samples were compared and t-paired or Wilcoxon tests when sample data was dependent. Qualitative data were analyzed by Chi-Square or Fisher tests. For correlation studies Pearson or Spearman tests were performed. Statistical significance among variables was defined as $p<0.05$. Prism 5 software package (GraphPad, USA) was used for analysis and graphical representation.

RESULTS

Baseline Characteristics of Individuals with Ckd

Table 1 shows patients' epidemiological profiles, which was composed of 25 adults and elderly people (66 ± 10 years) of both genders (12 women and 13 men). CKD was predominantly caused by type II DM (48%) or SAH (32%). The results showed no differences in the proportion between genders related to the main causes of CKD, SAH (female=3; male=5) or DM-II (female=7; male=4) (Fisher, p=0.369). The HD time for the study group was 4.7 ± 4.9 years until conversion to HDF; venous access was predominantly by arteriovenous fistula (60%), followed by long-term catheter (36%).

Table 1: Baseline characteristics of individuals with chronical kidney diseases

	Nº	Age (Years)	Time of hemodialysis (Years)	Etiology of chronic kidney disease	Vascular access
Female	1	60	7	Diabetes Mellitus - II	Long-term cateter
	2	80	2		
	3	67	10		Arteriovenous fistula
	4	73	0		
	5	67	2		Long-term cateter
	6	84	0		
	7	79	1		
	8	47	5	Immunopathogenesis	
	9	65	12	Genetics	
	10	53	20	Systemic arterial hypertension	Arteriovenous fistula
	11	78	1		
	12	63	1		
	13	66	2	Metabolic diseases	Polytetrafluoroethylene vascular prosthesis
	14	80	3		
male	15	55	4	Diabetes Mellitus - II	Arteriovenous fistula
	16	63	7		
	17	71	2		Long-term cateter
	18	61	11		
	19	62	8		
	20	73	4		
	21	62	0	Systemic arterial hypertension	Arteriovenous fistula
	22	68	1		
	23	66	4		
	24	42	1	Immunopathogenesis	Long-term cateter
	25	73	11	Chronic pyelonephritis	Arteriovenous fistula
	Median (Min to Max)	66 (42 to 84)	3 (0 to 20)	dvd	dvd

The results showed no difference in the proportion between genders related to the main causes of CKD, SAH (Female=3; Male=5) or DM-II (Female=7; Male=4) (Fisher, p=0.369). In addition, there was no difference in HD time between women and

men (t test, p>0.05); however, there was a negative correlation between HD time and the age of the individuals, that is, elderly individuals remained on HD longer (Spearman=0.017).

Evaluation of Haematological and Biochemical Parameters

Table 2 shows the results obtained for hematological and biochemical parameters.

CKD patient's erythrocyte parameters (hematocrit, hemoglobin, and Volume of Mean Corpuscular Hematocrit (VMC), Mean Corpuscular Hematocrit (HCM) and Range of Distribution of Erythrocytes (RDW)) were evaluated considering SAH and DM comorbidities and HD to HDF treatment conversion. There was a significant increase in hematocrit and hemoglobin in the DM group after treatment conversion (p=0.032). Hemoglobin levels were reduced after treatment conversion on SAH patients (p=0.039). Other erythrocyte parameters did not present a significant difference between groups. Leukocyte and platelet parameters in CDK patients with SAH and DM also did not suffer statistical difference after treatment conversion. Erythropoietic parameters and resistance index to erythropoietin action of pa-

Table 2: Hematological and biochemical data of the individuals with chronic renal diseases in HD, before and after 12 weeks of the convertion to HDF

	Cells and serum markers	HD	HDF	Units	Statistical
Leucocytes	Total of cells	7 (4 to 12)	7 (3 to 13)	mm3 x 103	p>0.05
	Granulocytes	3 (2 to 9)	4 (2 to 10)		
	Lynphocytes	2 (1 to 6)	1 (1 to 2)		
	Monocytes	7 (4 to 11)	6 (3 to 9)		
	Platellets	19 (12 to 36)	19 (9 to 39)	µL x 104	
Erithropoiesis	Erythropoietin	40 (8 to 104)	18 (1 to 78)	UL x 103	Pared t test p=0.008
	Erythropoietin resistance index	9 (0 to 35)	5 (0 to 25)	-	Pared t test p=0.021
Metabolism markers	Glucose	122 (76 to 246)	111 (80 to 263)		Wilcoxon p=0.049
	Triglycerides (TG)	165 (73 to 309)	163 (93 to 351)		p>0.05
	Cholesterol total (TC)	149 (110 to 224)	160 (113 to 217)		
	High density lipoprotein (HDL)	43 (24 to 66)	43 (27 to 74)		
	Low density lipoprotein (LDL)	84 (38 to 144)	88 (37 to 144)		
	Very Low density lipoprotein (VLDL)	113 (67 to 196)	116 (62 to 181)		
Ionic markers	HCO_{3-}	25 (21 to 28)	24 (22 to 28)	mEk/L	p>0.05
	Na^{+}	14 (13 to 14)	14 (13 to 15)		
	K^{+}	5 (4 to 6)	5 (4 to 7)		
	Mg^{2+}	2 (2 to 3)	2 (2 to 3)		
	PO_4^{3-}	4 (3 to 7)	5 (3 to 6)		
	Ca^{2+}	9 (8 to 10)	9 (8 to 14)		
Nutritional markers	Vitamin D	3 (1 to 6)	3 (2 to 5)	ng/mL	p>0.05
	Albumin	4 (4 to 5)	4 (4 to 5)	g/dL	
Toxicity markers	Aspartato aminotransferase (AST)	16 (9 to 27)	16 (9 to 24)	U/L	Wilcoxon p=0.049
	Alanina aminotransferase (ALT)	15 (9 to 25)	14 (9 to 27)		p>0.05
	Ferritin	339 (116 to 959)	316 50 to 903)	ng/mL	p>0.05
	Parathyroid hormone (PTH)	194 (41 to 831)	239 (32 to 702)	pg/mL	
	Alkaline phosphatase (ALP)	93 (49 to 163)	104 (67 to 162)	U/L	Wilcoxon p=0.026
	β2-Microglobulin (β2M)	28 (12 to 43)	24 (12 to 34)	µg/mL	Pared t test p=0.011
	C-Reactive Protein (CRP)	2 (1 to 5)	4 (1 to 15)	mg/mL	Wilcoxon p=0.038

HD-Hemodialysis; HDF-Hemodiafiltratin

tients submitted to HD to HDF treatment conversion can be observed. Data show significant reduction of EPO and resistance index to EPO on HDF patients (p=0.008).

Biochemical and metabolic parameters were also evaluated. Glucose levels were significantly reduced after HD to HDF treatment conversion (Wilcoxon p=0.049). Triglycerides, total cholesterol, HDL, LDL and NLDL did not show significant difference after treatment conversion (p<0.05). The glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) serum levels were also measured. Panel A, shows a significant reduction on GOT levels in HDF patients (Wilcoxon, p=0,049). GPT levels did not present any statistical difference among groups. Alkaline phosphatase (ALP) and C-reactive Protein (CRP) levels of CKD patients submitted to treatment conversion were increased (Wilcoxon, p<0.05), differently of β2-microglobulin levels, whose values were significantly decreased (t-paired test p<0.05). Ferritin and parathormon values did not show significant changes on patients submitted to treatment conversion.

Evaluation of Phagocytic Capacity and Production of Lipid Bodies and Cytokines

Table 3 shows the results of phagocytic capacity, lipid bodies (Table 4) and cytokines (Table 5) production for individuals with CKD after treatment conversion from HD to HDF. As we evaluated phagocytosis index (PI) by comparing 5 or 20 yeasts/cell, it was lower with HD treatment compared to HDF and the control (Kruskal–Wallis, p<0.01). This result was due to the lower recruitment of monocytes for phagocytosis for both receptors (Kruskal–Wallis, p<0.001) but also due to the higher number

Table 3: Phagocytic capacity of macrophages production of lipid corpuscle by leukocytes

Pagocytosis by macrophages	5 yeats / cell				20 yeasts/cell			
	Median (mim to max)			Test p value	Median (mim to max)			Test p value
	Control	HD	HDF		Control	HD	HDF	
% Cells involved in phagocytosis	12 (9 to 21)	2* (1 to 5)	6 (1 to 12)	KW p<0.001	61 (47 to 89)	2* (1 to 4)	5* (2 to 21)	AN p<0.001
Number of yests ingested by cell	1.7 (1.0 to 2.1)	1.0* (0.5 to 1.3)	1.3 (1.2 to 2.5)	KW p=0.001	1.6 (1.3 to 2.0)	1.0* (1.0 to 1.6)	1.6 (1.0 to 2.2)	KW p=0.036
Phagocytic index	18 (14 to 37)	2* (1 to 6)	9 (2 to 14)	KW p<0.001	92 (66 to 119)	3* (1 to 6)	9 (2 to 39)	AN p<0.001
% Cells involved in phagocytosis	57 (47 to 89)	9* (6 to 21)	32* (13 to 45)	AN p<0.001	70 (54 to 87)	10* (5 to 16)	39* (12 to 44)	AN p<0.001
Number of yests ingested by cell	1.6 (1.3 to 2.0)	1.4 (1.0 to 2.5)	1.9* (1.3 to 3.0)	KW p=0.027	1.7 (1.5 to 2.1)	1.7 (1.0 to 4.1)	2.5* (1.8 to 3.8)	AN p=0.004
Phagocytic index	85 (66 to 126)	11* (6 to 32)	56 (25 to 134)	KW p=0.001	115 (81 to 183)	17* (6 to 39)	97 (32 to 166)	AN p=0.001

Table 4: Production of cytokines

Lipid Corpuscle by leukocytes	Basal production (non-stimuletad)				Stimulated production (yeast + individual serum)			
	Mean ± SD			ANOVA p value	Mean ± SD			ANOVA p value
	Control	HD	HDF		Control	HD	HDF	
% Cells with lipid corpuscle	89 ± 7	95 ± 3	92 ± 6	p>0.05	96 ± 3	95 ± 7	97 ± 3	p>0.05
Number of lipid corpuscle/celll	5 ± 1	5 ± 1	4 ± 0		8 ± 2	9 ± 2*	7 ± 2	p=0.018
Corpuscular index	444 ± 92	448 ± 67	395 ± 45		796 ± 156	814 ± 275	635 ± 151	p=0.040

Table 5: CKD treated with HD and 12 weeks after conversion to HDF

Production of cytokines	Cytokines (pg/mL)	Control	HD	HDF	Test p value
By Macrophages		Median (mim to max) (%)			
Inflamatory	Interleucin-2	0.9 (0.8 to 1.7)	0.8 (0.6 to 1.8)	0.8 (0.7 to 1.3)	AN p>0.05
	Interleucin-6	0.3 (0.0 to 1.7)	3.3* (0.0 to 55.0)	4.6* (0.8 to 41.9)	KW p=0.001
	Interleucin-17A	3.1 (2.7 to 4.6)	3.5 (0.5 to 11.4)	3.2 (1.5 to 13.6)	KW p>0.05
	Fator de necrose tumoral	0.0 (0.0 to 4.2)	0.0 (0.0 to 2.4)	0.1 (0.0 to 20.3)	KW p=0.028

Anti-inflamatory	Interleucin-4	0.8 (0.3 to 0.9)	0.4* (0.0 to 1.0)	0.5 (0.2 to 1.3)	KW p=0.016
	Interleucin-10	1.4 (1.3 to 1.6)	1.6 (0.9 to 34.0)	1.6 (1.0 to 12.0)	
By lymphocytes	Interferon-γ	1.0 (0.8 to 1.0)	1. (0.7 to 1.5)	1.1 (0.8 to 2.0)	KW p>0.05

Table 6: Production of superoxide reactive oxygen species by leukocytes of individuals with CKD treated with HD and 12 weeks after conversion to HDF

Production of Superoxide (O_2)	Percentage of cells that:	Control	HD	HDF	Test p value
		Median (mim to max) (%)			
Basal production (cells without yeasts)	produced O_2	37 (19 to 71)	20* (4 to 33)	54 (10 to73)	AN p=0.003
Stimulated production (cells+yeasts)	ingested yeasts and produced O_2	73 (50 to 81)	9* (5 to 21)	52* (17 to82)	AN p<0.0001
	did not ingested yeasts, but produced O_2	17 (7 to 46)	5* (0 to 10)	14 (2to33)	KW p=0.007
	ingested yeasts and did not produce O_2	17 (7 to 46)	5* (0 to 10)	14* (2to33)	KW p<0.0001
	did not ingested yeasts and did not produce O_2-	4 (1 to 7)	23* (7 to 52)	5 (0to32)	KW p<0.0001

of ingested yeasts in mediated phagocytosis (Kruskal–Wallis, p=0.001). This indicates that the treatment conversion did not affect PI (**Table 6**).

The basal production of lipid bodies presented no significant difference among the three groups (control, HD and HDF treatment; ANOVA, p>0.05). However, when cells were stimulated, there was an increase on lipid bodies production on HD treated patients compared to the control and HDF (ANOVA, p=0.018). In addition, HDF reduced the stimulated corpuscular index when compared to HD (ANOVA, p=0.040).

Levels of IL-2, IL-10, IL-17 and INF-γ did not differ among control, HD and HDF groups. However, there was a higher production of IL-6 in HD and HDF compared to the control (Kruskal–Wallis, p=0.001) and lower production of IL-4 in HD compared to the control (Kruskal–Wallis, p=0.016). In addition, IL-4 and TNF production was higher in HDF than in HD (Kruskal–Wallis, p<0.05).

Evaluation of Superoxide (O_2) Production

Table 4 shows the percentage of cells reacting to O_2 production. Baseline production of O_2 was lower in the HD group than in the control and HDF groups (ANOVA, p=0.003). In addition, it was evaluated the combination effect of phagocitation and O_2 production, and the results were also presented **Figure 1**.

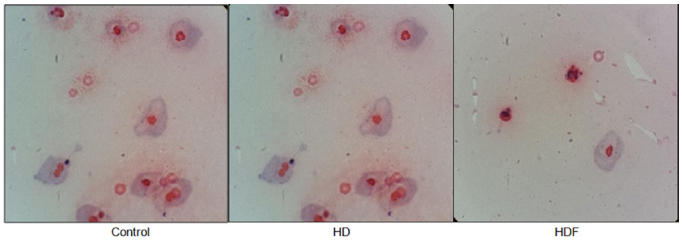

Figure 1: Circle indicates a phagocyte that ingested yeasts, but did not produce O_2●. Arrows indicate phagocytes that not ingested yeasts, but produced O_2●. Stain with Giemsa (10%). 1000 x of magnification

DISCUSSION

This study evaluated the HDF effects on several Brazilian CKD patient's parameters. Despite the widespread use of HD treatment in Brazil, literature has consistent evidence of benefits of applying HDF technique on CKD individuals (9-11). We observed, despite the reduced patient sample, that DM was the prevalent etiology among patients. This data corroborates findings of other studies, which point out DM as the main comorbidity associated with CKD [12,13].

A promising result brought by the HDF treatment conversion is the reduction of glycemic values. As DM is the main comorbidity associated with kidney disease, the reduced glucose levels indicates a possible control of the disease, resulting in better clinical conditions for the patients. Hemoglobin and hematocrit values were significantly reduced on DM patients of this study, being important tools for these individuals against thrombosis and stroke risks [14,15].

Our results evidenced the EPO resistance index reduction on HDF patients. This index is directly correlated with chronic inflammation in CKD patients because of metabolic alterations on cytokine-mediated erythropoiesis, such as IL-1, TNF-alpha and IFN-gamma [16,17]. Previous studies have demonstrated that HDF technique is associated with a reduction of chronic inflammation by depurating larger molecules, such as pro-inflammatory cytokines and other molecules, as EPO [9,16-19].

HDF treatment conversion also affected β2-microglobulin, GOT, ALF and CRP levels. β 2-microglobulin and GOT values diminished, while ALF and CRP levels increased significantly. The decrease in the levels of the mentioned markers corroborates the studies that show that the medium weight molecules clearance in HDF is responsible for reducing inflammatory processes [9-11]. The type of dialytic method does not affect clearance of low molecular weight molecules (10). B2-microglobulin levels are increased 4 to 7 times on CKD patients [20,21]. Therefore, the HDF treatment conversion promoted the significantly reduced levels of this inflammatory marker, indicating that medium weight solutes depuration can be favorable for clinical patients' condition [22-24]. The hepatic enzyme GOT, also described as aminotransferase aspartate (AST), has also its levels reduced by the dialytic treatment conversion. Altered levels

of this enzyme were associated with higher mortality rates of CKD patients [25]. However, some studies describe that CKD patients undergoing HD treatment have lower GOT values than healthy individuals [26,27]. It was related that HDF treatment conversion also did not affect GOT levels [28]. Therefore, more evidence is needed on the HDF effect in this liver damage marker.

Our data also indicated increased ALP levels. This enzyme hydrolyses inorganic phosphate, which is a potent endogenous inhibitor of calcification. This is also an important cardiovascular risk marker investigated on kidney patients, especially the old-aged ones [29-31]. As elderly individuals constituted our patients sample, this is an important marker to be observed.

Serum levels of calcium, phosphorus, PTH and ALP, as well as other markers involved in bone metabolism, are altered on CKD. These conditions lead to worse kidney functions, as well as lower vitamin D levels and loss of bone mineral density [32]. Higher levels of ALP alone cannot indicate clinical restriction for HDF use on CKD patients, because PTH, phosphorus, calcium, vitamin D and magnesium levels were not altered.

Another change observed on patients undergoing HDF treatment was the increase in CRP levels. This liver protein is one of the main inflammatory markers, especially associated with CKD [9,33]. Differently from what it was observed in this study, the literature dealing with the parameters of patients for whom the treatment was converted from HD to HDF has indicated a reduction in serum levels of CRP [11,19,34]. However, it should be noted that this marker alone cannot be assessed as an indication of an imminent state of ongoing inflammation. Analyzing the quantification of the patients' cytokines, for example, it appears that the values of IL-10 and IL-6, which are cytokines, related to the inflammatory process in conjunction with other markers, did not change between HD and HDF groups, only compared to the control (healthy individuals). In addition, it is important to highlight that there are several parameters described for the application of HDF [9,35]. Therefore, considering that this study was carried out with a 3 month follow-up in a relatively small sample group, further adjustments in the technique can still be made to optimize it aiming at improving the levels of inflammatory markers in patients.

HDF treatment conversion also influenced the monocyte's phagocytic capacity by increasing recruitment for opsonin-mediated phagocytosis, thereby increasing the phagocytic index. When phagocytosis was evaluated by pathogen receptors, there was no significant difference in the phagocytic index due to the conversion of the dialysis technique.

HDF has been associated with a decrease in the inflammatory state in CKD and an improvement in the function of granulocytes (neutrophils) [7]. Studies show that several granulocyte inhibitory proteins that are retained in uremic patients, and that end up contributing to a higher incidence of infections, are cleared by the HDF technique [36]. This explains the significant increase in the phagocytic index observed in the application of HDF.

Additionally, by correlating the increase in the phagocytic index in opsonin-mediated phagocytosis with the increase in CRP levels, it can be inferred that CRP, by acting on innate immunity as an opsonin, is indirectly involved on this increase in the phagocytic index observed in patients treated with HDF. This study also quantitatively evaluated the proportion of lipid bodies per cell by comparing the groups of dialysis patients and healthy patients groups. HDF significantly decreased the corpuscular index in comparison with HD, showing values similar to those of the control group. This difference can be justified by the inhibition of inflammatory processes that were previously activated by CKD itself and also by the dialysis method (HD) [14].

This work also quantified a series of inflammatory and anti-inflammatory cytokines in patients undergoing HD and HDF, both for comparison with a control group of healthy individuals and for assessing the individual effect of HDF by analyzing each patient who consented to convert his treatment from HD to HDF. Data show there was only an increase in the production of the inflammatory cytokine TNF-α and in the anti-inflammatory cytokine IL-4. In these measures, the high standard deviation presented in the IL-6 and IL-10 values stood out, precisely the main pro and anti-inflammatory cytokines studied in CKD and in its dialysis treatments [7,14,37,38]. Thus, in view of the reduced number of patients evaluated in this study and considering that possible external situations that affect the cytokines measurement in these patients were not excluded, future assessments can be made to refine the data In addition, there are studies in the literature that also reported unchanged cytokine values such as IL-10 and IL-6 when comparing dialysis techniques [38]. This fact alone can also be just an indication of the safety of HDF for the treatment of patients with CKD, since it would not significantly alter parameters as sensitive as these inflammatory markers in patients who are already in a pathological process.

In the experiment using the methodology of the paired samples, that is, in the measurement of the cytokine levels of the same individual who was previously treated with HD and who converted his treatment to HDF, before and after the treatment change, a slight reduction in the mean IL-10 and increases in IL-17A and TNF-α levels. IL-17A stimulates the production of other inflammatory cytokines, such as IL-6 itself, which, despite not showing altered values in this study, showed great variations among patients. TNF-α, among other functions, is also responsible for activating phagocytic cells. This data can probably be correlated with the increase in the phagocytic index, which was also observed in this study.

CONCLUSION

In conclusion, HDF has established itself as a safe and efficient technique for the clearance of medium molecular weight molecules without prejudice to the patient and significantly improves several inflammatory markers that are altered not only by pathology. This research brings about the effects on immune parameters of kidney patients undergoing treatment conversion of hemodialysis to hemodiafiltration. Hemodiafiltration is not a usual treatment in Brazil, so this study can promote the benefits of changing the therapeutic approach for the patients and the public health system. Finally, this work presented several data that corroborates the literature which indicates HDF as a technique that enhances clinical, biochemical and inflammatory markers on CKD patients.

AUTHOR CONTRIBUTION

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DISCLOSURES

All authors have nothing to disclose.

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