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Chronobiological Changes in Biochemical Hemorrheological Parameters and Cytokine Levels in the Blood of Diabetic Patients on Hemodialysis: A Cross-Sectional Study

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Abstract: Introduction: Diabetes mellitus, a chronic disease characterized by hyperglycemia, is a significant contributor to chronic kidney disease, particularly in patients with diabetic nephropathy undergoing renal replacement therapy. Variations in circadian rhythms can influence the progression of chronic diseases and treatment outcomes. **Aims:** This observational study evaluated gender-based chronobiological changes in biochemical, hemorheological factors, and cytokines in patients with dialysis-dependent diabetic nephropathy. **Materials and Methods**: A cross-sectional study was conducted in Barra do Garças, Brazil, involving 46 patients with type 2 diabetes mellitus who were on regular hemodialysis. Participants were divided into four groups for analysis according to gender and period of day. Inclusion criteria included individuals with type 2 diabetes mellitus undergoing periodic hemodialysis, receiving hemodialysis care at the service, and signing a consent form. Patients with an age under 18 years, diabetes mellitus type 1, or with autoimmune diseases were excluded. Blood samples were collected to assess melatonin, cortisol, biochemical and hemorheological parameters, and cytokines such as IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, and IL-17. **Results**: Men exhibited higher melatonin, urea, and creatinine levels in the morning and afternoon phases. At the same time, women showed lower melatonin, increased viscosity, and a decreased deformation rate in the afternoon. Additionally, levels of TNF-α, IFN-γ, and IL-17 were lower in morning serum samples from women. **Conclusion:** These findings suggest that both gender and circadian timing should be taken into account, when optimizing hemodialysis treatment for patients with diabetic nephropathy. A deeper understanding of these factors could lead to more personalized and effective treatment strategies, ultimately improving patient outcomes and enhancing their quality of life.

Keywords: diabetes mellitus; diabetic nephropathy; cytokines; chronic renal disease; rheology

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1. Introduction

Diabetes mellitus is characterized by hyperglycemia and has been linked as a major cause of end-stage renal failure, significantly impacting public health. The increasing prevalence of obesity and population aging have led to a sharp increase in cases of the disease [1]. Despite advances in diabetes treatment, diabetic nephropathy remains a prevalent complication and a primary cause of chronic kidney disease [2].

Renal alterations in diabetic patients result from the direct and indirect effects of glucose, which contribute to the pathogenesis of diabetic nephropathy through mechanisms such as oxidative stress and the secretion of specific proteins. These processes can lead to chronic renal failure [3], where subclinical inflammation plays a critical role in disease progression, with elevated plasma levels of acute phase proteins such as C-reactive protein (CRP) and several cytokines, including IL-1, IL-6, IL-8, TNF-α, and TGF-β [4,5].

Cytokines such as TNF-α and IL-1, in addition to promoting ferritin expression and iron storage within macrophages, also inhibit erythropoietin (EPO) production by the kidneys, leading to anemia due to reduced activity of erythrocyte progenitor cells [6,7]. Biochemical assessment in patients with chronic kidney disease is crucial since abnormalities such as elevated serum creatinine and urea levels occur, along with changes in hemoglobin, phosphorus, potassium, calcium, and albumin levels [8]. Although hemodialysis effectively manages chronic kidney disease, it presents challenges, particularly in producing erythropoietin required for erythropoiesis [9].

Daily circadian and behavioral rhythms aligned with environmental cycles influence several bodily functions, including metabolism, immune responses, and hormone secretion [10,11]. Furthermore, studies indicate that factors such as hematocrit, blood viscosity, red blood cell deformation, and aggregation impact blood rheology [12]. However, there is a notable lack of studies focusing on hemorheological changes in diabetic patients undergoing hemodialysis [13–15].

Diabetic patients generally start dialysis earlier than those with other renal conditions due to a more rapid decline in renal function, often accompanied by malnutrition, anemia, and fluid retention [16]. Chronobiological changes may also correlate with the onset of metabolic diseases such as diabetes, which can lead to irreversible damage in diabetic nephropathy [17–19]. Melatonin and cortisol, key regulators of circadian rhythms, play crucial roles in maintaining homeostasis and are often disrupted in metabolic diseases such as diabetes, potentially exacerbating complications like diabetic nephropathy [20–22].

This study aims to explore the chronobiological changes in hemorheological and biochemical parameters and cytokine levels in the blood of patients with type 2 diabetes and diabetic nephropathy undergoing hemodialysis treatment at different times of the day.

2. Materials and Methods

2.1. Study Design

A cross-sectional study evaluated the hemorheological and biochemical parameters and cytokine profile in patients with chronic kidney disease. The subjects attended the Nephrology Institute of Araguaia in Barra do Garças, MT, Brazil. The patients were recruited in the years of 2022 and 2023. The Institutional Research Ethics Committee approved this study, and all the subjects gave informed written consent before entering the experimental protocol.

2.2. Inclusion and Exclusion Criteria

The inclusion criteria were as follows: (a) individuals with type 2 diabetes mellitus who were undergoing periodic hemodialysis; (b) received hemodialysis care at the service;

and (c) signed a consent form. Patients with an age under 18 years, diabetes mellitus type 1, or with autoimmune diseases were excluded.

2.3. Participants of Study

In this study, 46 patients with chronic kidney disease and type II diabetes mellitus were categorized into four groups based on gender and the timing of their hemodialysis sessions: Male Morning (n = 10), Female Morning (n = 10), Male Afternoon (n = 15), and Female Afternoon (n = 11). This classification was developed to observe potential chronobiological variations related to gender and dialysis period.

Due to the specific characteristics of the target population and the inclusion criteria, a convenience sampling approach was employed. The sample size proposed in this study was based on statistical calculations of the sample size assuming a loss to follow-up of around 10% and correcting for the effects of errors α (5%) and β (20%) attributed to the study, where the minimum sample size per group would be 8 individuals.

This adjustment ensured that the study was both feasible and ethically appropriate.

Before the study commenced, the patients were thoroughly informed about the research. They received clear and comprehensive verbal and written information regarding the study's objectives and significance. All participants subsequently signed the informed consent form.

2.4. Sample Collection

Blood samples were collected from patients with chronic kidney disease and type II diabetes mellitus to examine chronobiological variations in hemorheological and biochemical parameters, as well as cytokine profile. The collections were made through venipuncture before patients were submitted to hemodialysis, in the morning at 8:00–10:00 h and the afternoon at 14:00–16:00 h, following the protocol developed by França et al. [20]. Blood was collected by venipuncture, with approximately 10 mL for each sample, and then placed in Vacutainer tubes with EDTA (Beckton Dickinson®, Franklin Lakes, NJ, USA) and tubes with clot activator to obtain serum (Beckton Dickinson). The EDTA samples were subjected to hematological and rheological evaluation on the same collection day. The serum was obtained by centrifugation for 10 min at 160× g and stored at −80 °C until biochemical and cytokine analysis.

2.5. Melatonin and Cortisol Determination

Melatonin was extracted from serum and quantified using a commercial ELISA kit from Immuno-Biological Laboratories (IBL, Hamburg, Germany). The kit features include a lower detection limit of 1.6 pg/mL, with intra-assay and inter-assay coefficients of variation ranging from 3.0% to 11.4% and 6.4% to 19.3%, respectively.

Melatonin was extracted using the affinity chromatography method with standardized columns. After melatonin extraction, each sample, standard, and control were placed on an ELISA plate and analyzed according to the manufacturer's instructions. Readings were taken using a plate spectrophotometer set at 405 nm. Results were derived from a standard curve and expressed in pg/mL.

Serum cortisol levels were quantified using a commercial microplate ELISA test from Kit DRG® International, Inc. (Springfield, NJ, USA). The test has a lower detection limit of 100 pg/mL, with intra-assay and inter-assay coefficients of variation of 8.1% and 8.8%, respectively. Each standard, control, and serum was placed onto an ELISA plate and analyzed according to the manufacturer's instructions. Readings were taken using a plate spectrophotometer set at 450 nm. Results were determined using a standard curve and expressed in ng/mL.

2.6. Biochemical Determination

The dosages for glucose, urea, creatinine, and Glutamic-Pyruvic Transaminase (GPT) in serum were determined with commercial kits of the brand Biosystems® (Recife, Pernambuco, Brazil) using the enzymatic and kinetic colorimetric methodology and the automated equipment of biochemistry A15 Biosystems® (Recife, Pernambuco, Brazil). A Celm® flame spectrophotometer (São Caetano do Sul, São Paulo, Brazil) was used according to the manufacturer's instructions to determine potassium.

2.7. Hematological Analysis

In the hematological evaluation, an automated ABX Petra 60 Horiba® analyzer (Osaka, Kansai, Japan) measured hemoglobin, determined hematocrit, and quantified platelets using cytochemistry and an impedance methodology.

2.8. Hemorrheological Analysis

For rheological analysis, 2 mL of whole blood was collected from each patient in hemodialysis maintenance vacuum tubes with EDTA anticoagulant. The analyses were performed on a Modular Compact Rheometer (Anton Paar® GmbH, Ostfildern, Germany—MCR 102) coupled with the Rheoplus V3.61 software. Permanent control of the measurement gap was provided by 0.099 mm TruGap™ support, a Toolmaster™ CP 50 measurement cell, and precise temperature control with the T-Ready™ feature. In all analyses, 600 μL of blood was added to the surface of the reading plate.

Parameters were established for the flow curves and viscosity, with the control of shear stress [τ] at 0–5 Pa for the upsweep and 5–0 Pa for downward curves; tests were performed at 37 °C, with 75 readings analyzed. For the viscosity curve, the parameters were based on the fixed control shear stress [τ], with 41 readings analyzed.

2.9. Cytokine Determination

The Cytometric Bead Array-Kit (CBA, BD Bioscience, San Jose, CA, USA) assessed the cytokine levels in serum from patients with chronic kidney disease. In this study, cytokines, IFN-γ, TNF-α, IL-2, IL-4, IL-6, IL-10, and IL-17 were quantified in serum samples. These cytokines were analyzed using flow cytometry (FACSCalibur, BD Bioscience). Before analyzing the cytokines, the flow cytometer settings were verified by following the manufacturer's instructions and using tracking beads (BD Calibrite™ 3 Beads, BD Bioscience). Compensation beads were then used to establish compensation settings in FACSComp™ software on Mac® OS 9 (BD Biosciences, San Jose, CA, USA). The same compensation matrix was applied to all samples to ensure high accuracy and reliability in our methodology. Data were analyzed using the FCAP Array software V.3.0 (BD Bioscience).

2.10. Statistical Analysis

The data were organized in Excel@ and are presented as the mean ± standard deviation (SD). Statistically significant differences in biochemical, hematological, and rheological parameters and cytokine levels were assessed with the BioEstat® version 5.0 software [Mamirauá Institute, Belém, Brazil]. A D'Agostino normality test and two-way analysis of variance (ANOVA) were used to analyze the data statistically. Correlation analysis used Pearson's linear correlation test. Statistical significance was considered for a p-value less than 0.05 ($p < 0.05$).

3. Results

3.1. General Characteristics of Patients

In this study, 46 chronic renal patients with type II diabetes mellitus who were undergoing regular hemodialysis treatment were evaluated. In total, 54% were male, and 46%

were female. The mean age of the patients was 5.4 ± 5.1 years. The male patients had a mean age of 67.1 ± 4.0, while the female patients had a mean age of 64.1 ± 5.3; 13% were smokers, 21.6% were ex-smokers, and 10.8% had hypertension (Table 1).

Table 1. General characteristics of patients who underwent hemodialysis.

Parameters	Male	Female	Patients
Gender	54% (*n* = 25)	46% (*n* = 21)	100% (*n* = 46)
Age (years)	67.1 ± 4.0	64.1 ± 5.3	65.4 ± 5.1
Smokers	6.5% (*n* = 4)	4.3% (*n* = 2)	13.0% (*n* = 6)
Ex-smokers	13.5% (*n* = 5)	8.1% (*n* = 3)	21.6% (*n* = 8)
Hypertension	6.5% (*n* = 3)	5.5% (*n* = 2)	10.8% (*n* = 5)

The data were expressed in percentages and numbers (*n*) or the mean and standard error.

3.2. Melatonin and Cortisol Analysis

Melatonin levels were higher in the serum of diabetic males undergoing hemodialysis in the morning compared to diabetic females in the same period (see Figure 1A). In the afternoon, however, the melatonin levels in diabetic males undergoing hemodialysis were lower than those measured in the morning (Figure 1A).

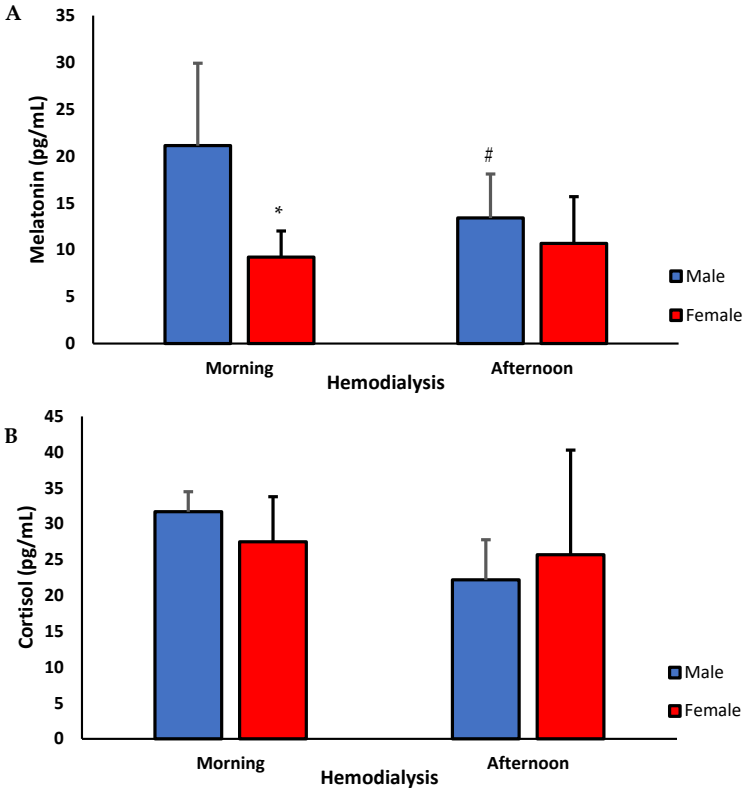

Figure 1. Melatonin (pg/mL) (**A**) and cortisol (ng/mL) (**B**) concentrations in serum from diabetic patients who underwent hemodialysis at different times of day (morning or afternoon). Data are expressed as mean and standard error. Melatonin: F(1,36) = 0.5230; p = 0.5156 (time of day); F (1,36) = 5.0598; p = 0.0340 (gender); F (1,36) = 2.1712; p = 0.1531 (interaction). Cortisol: F (1,36) = 0.8417; p = 0.6271 (time of day); F (1,36) = 0.2556; p = 0.6240 (gender); F (1,36) = 3.7191; p = 0.0452 (interaction). p-values were obtained from two-way ANOVA analyses. * comparing the male and female, considering the same period of the day. # comparing the period (morning and afternoon), considering the same sex.

Regardless of gender or the time of day during hemodialysis, cortisol hormone levels were similar across all groups studied. Nevertheless, an interaction between corti-

sol levels and other variables was observed, with a statistical result of F = 3.7191 and $p = 0.0452$ (Figure 1B).

3.3. Biochemical Parameters

In the analysis of biochemical parameters, it was observed that urea and creatinine levels were lower in diabetic women undergoing hemodialysis, independently of the phases of the day. The glucose, Glutamic-Pyruvic Transaminase (GPT), and potassium did not vary between collection periods (morning or afternoon) or between genders (Table 2).

Table 2. Biochemical parameters of male and female individuals who underwent hemodialysis at different collection periods (morning or afternoon).

Biochemical Parameters	Morning	Afternoon	Statistical
Urea (mg/dL)			F (1,36) = 0.5880; $p = 0.5433$ (time of day)
Male	138.0 ± 30.4	123.7 ± 24.0	F (1,36) = 8.2526; $p = 0.0082$ (gender)
Female	99.3 ± 21.4 *	110.0 ± 25.9 *	F (1,36) = 1.0211; $p = 0.3237$ (interaction)
Creatinine (mg/dL)			
Male	13.42 ± 1.2	12.80 ± 2.8	F (1,36) = 0.1738; $p = 0.6822$ (time of day)
Female	9.15 ± 2.62 *	7.35 ± 2.4 *	F (1,36) = 6.9088; $p = 0.0458$ (gender) F (1,36) = 2.9838; $p = 0.0617$ (interaction)
Glucose (mg/dL)			
Male	158.4 ± 66.01	174.8 ± 92.5	F (1,36) = 0.6203; $p = 0.5556$ (time of day)
Female	138.5 ± 25.80	202.7 ± 115.6	F (1,36) = 2.9469; $p = 0.0955$ (gender) F (1,36) = 0.0174; $p = 0.8913$ (interaction)
GPT (mg/dL)			
Male	19.7 ± 7.1	20.3 ± 17.9	F (1,36) = 0.5550; $p = 0.5301$ (time of day)
Female	22.0 ± 14.6	16.3 ± 6.4	F (1,36) = 0.6314; $p = 0.5597$ (gender) F (1,36) = 1.3048; $p = 0.2638$ (interaction)
Potassium (mmol/L)			
Male	5.7 ± 0.7	5.64 ± 0.85	F (1,36) = 2.3595; $p = 0.1342$ (time of day)
Female	4.8 ± 0.6	5.32 ± 0.66	F (1,36) = 0.8494; $p = 0.6312$ (gender) F (1,36) = 1.8144; $p = 0.1878$ (interaction)

The results represent the mean and standard error of 46 different hemodialysis patients. GPT (Glutamic-Pyruvic Transaminase). *p*-values were obtained from two-way ANOVA analyses. * comparing the male and female, considering the same collection period.

3.4. Hematological Parameters

Hematocrit and hemoglobin were lower in diabetic women undergoing hemodialysis in the morning compared to the levels shown in the serum of men undergoing hemodialysis in the same collection period. In diabetic women undergoing hemodialysis in the afternoon, the hematocrit increased to values similar to those found in male patients. The platelets were lower in diabetic men undergoing hemodialysis in the afternoon (Table 3).

Table 3. Hematological parameters of male and female individuals who underwent hemodialysis at different times (morning or afternoon).

Hematological Parameters	Morning	Afternoon	
Hematocrit (%)			
Male	27.1 ± 2.54	27.4 ± 2.15	F(1,36) = 8.1114; $p = 0.0087$ (time of day)
Female	22.57 ± 1.67 *	26.36 ± 5.33 #	F (1,36) = 10.612; $p = 0.0314$ (gender) F (1,36) = 0.8262; $p = 0.6243$ (interaction)
Hemoglobin (g/dL)			

Table 3. *Cont.*

Hematological Parameters	Morning	Afternoon	
Male	9.45 ± 1.03	10.02 ± 0.77	F (1,36) = 0.6887; p = 0.5802 (time of day)
Female	8.24 ± 0. 97 *	9.72 ± 1.82	F (1,36) = 6.1982; p = 0.0191 (gender) F (1,36) = 2.6607; p = 0.1124 (interaction)
Platelets (mm^3)			
Male	313. 857 ± 50.734	219.666 ± 52.902 *	F (1,36) = 4.0281; p = 0.0322 (time of day)
Female	258. 857 ± 89.063	241.181 ± 69.719	F (1,36) = 2.0451; p = 0.1625 (gender) F (1,36) = 4.2742; p = 0.0471 (interaction)

The results represent the mean and standard error of 46 different hemodialysis patients. p-values were obtained from two-way ANOVA analyses. * comparing the male and female, considering the same collection period; # comparing the collection period (morning and afternoon), considering the same sex.

3.5. Rheological Parameters

The blood flow curves of diabetic women who underwent hemodialysis in afternoon periods showed there was a lower shear rate (mean = 777.28) compared to women who underwent hemodialysis in the morning period (mean = 1033.52), indicating that in the morning period, it may result in greater cell deformation (Figure 2A). The blood flow curves of diabetic men who underwent hemodialysis were similar in both periods (Figure 2B). It can also be observed that independently of periods (morning and afternoon), the blood flow shifts showed a parabolic curve, which ascended non-linearly and was characteristic of non-Newtonian fluids (Figure 2A,B).

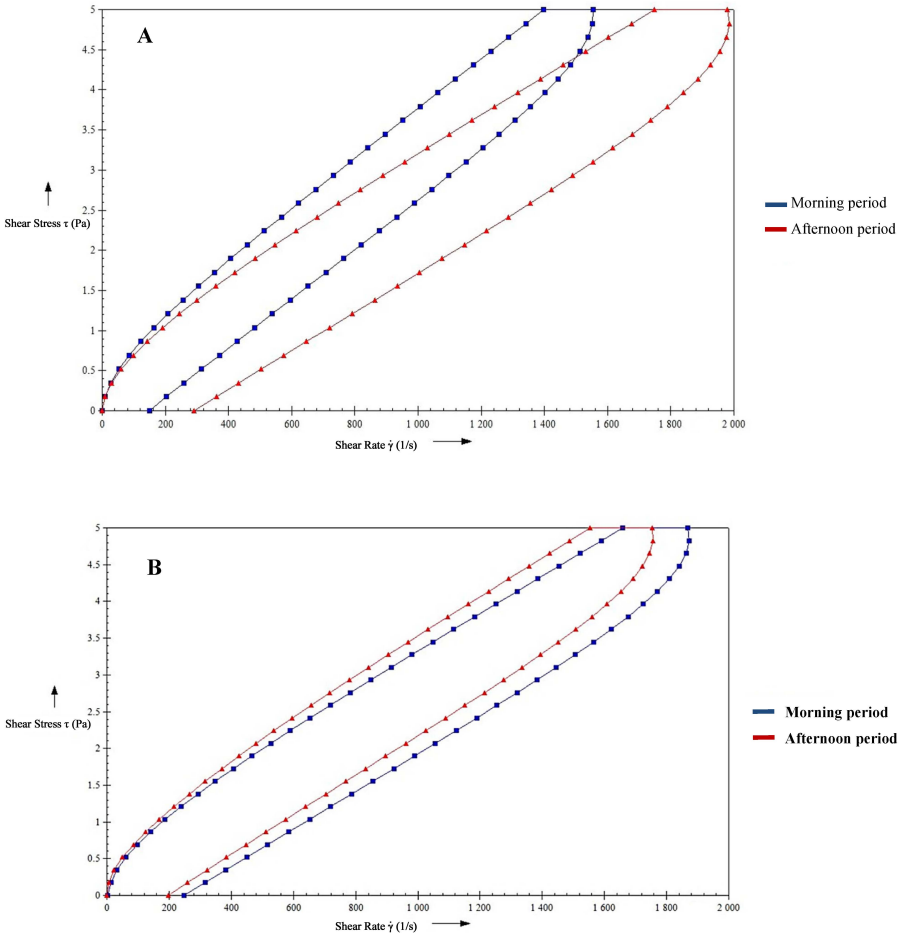

Figure 2. Blood flow curves of female (**A**) and male (**B**) diabetic patients undergoing hemodialysis in the morning and afternoon periods. The results represent the mean of 46 different hemodialysis patients. p-values were obtained from two-way ANOVA analyses. F (1,36) = 0.6320; p = 0.7516 (time of day); F (1,36) = 1.518; p = 0.3510 (gender); F (1,36) = 3.2722; p = 0.4301 (interaction).

The blood viscosity curves of diabetic women who underwent hemodialysis in the afternoon periods (mean = 0.00318) were higher ($p < 0.05$) compared to diabetic women who underwent hemodialysis in the morning periods (mean = 0.00236; Figure 3A). No significant difference in blood viscosity, regardless of period, was detected in diabetic men who underwent hemodialysis (Figure 3B).

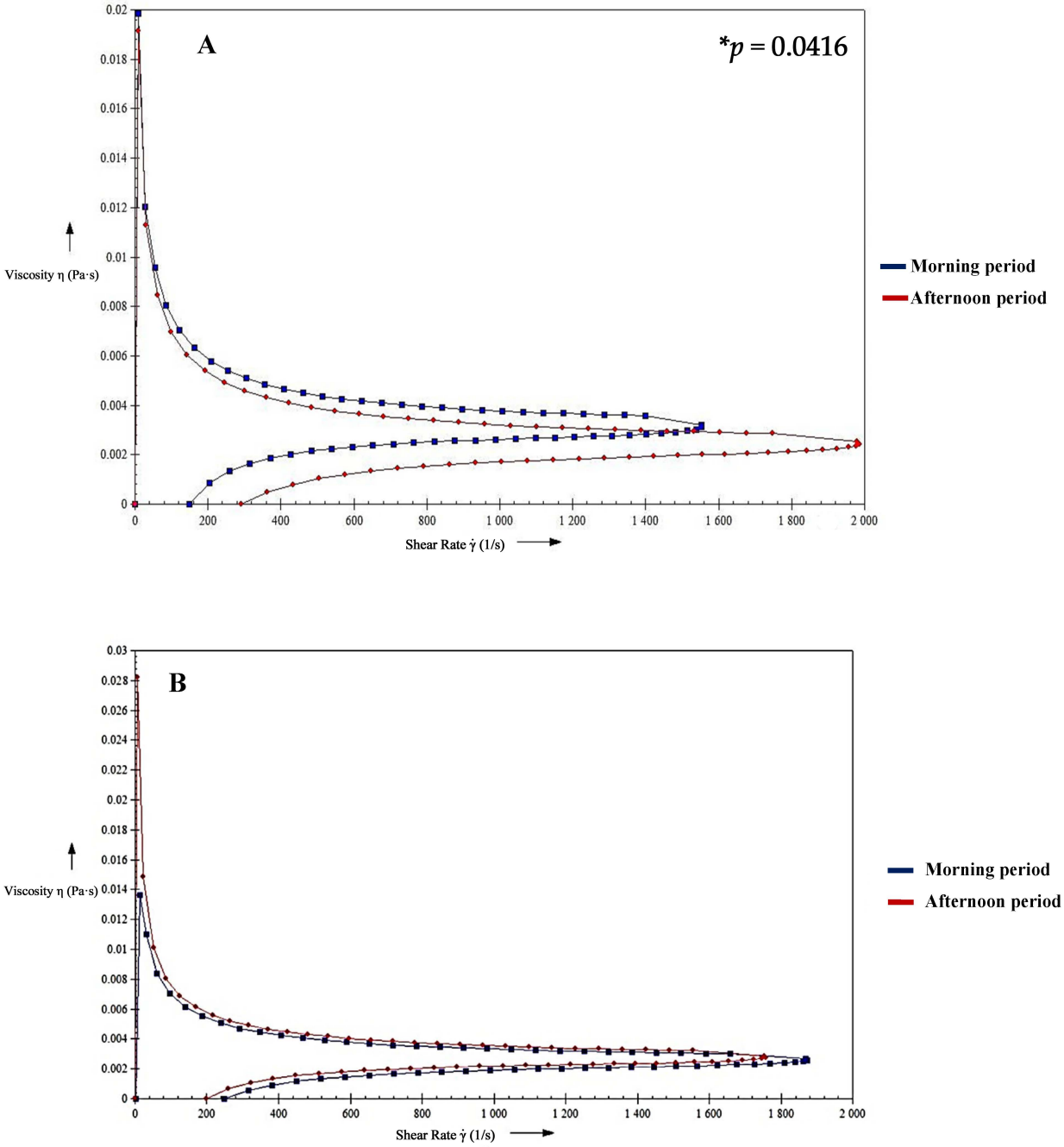

Figure 3. Viscosity curves of female (**A**) and male (**B**) diabetic patients undergoing hemodialysis in the morning and afternoon periods. The results represent the mean of 46 different hemodialysis patients. F (1,36) = 6.310; p = 0.0416 (time of day); F (1,36) = 0.327; p = 0.7213 (gender); F (1,36) = 1.733; p = 0.2751 (interaction). p-values were obtained from two-way ANOVA analyses. * statistically significant difference.

3.6. Cytokine Levels

Table 4 shows the cytokines in a patient who underwent hemodialysis in the morning and afternoon. In the morning, there was a reduction in TNF-α, IFN-γ, and IL-17 and

an increase in IL-10 in the afternoon in the serum of women compared to men. In the comparison between the periods, it is observed that IL-4 and IL2 increased in individuals of both sexes who underwent hemodialysis in the afternoon.

Table 4. Cytokine levels in serum of male and female patients with diabetic nephropathy undergoing hemodialysis in the morning and afternoon. The results represent the mean and standard error of the analysis of different hemodialysis patients.

	Morning		Afternoon		Statistical
Cytokines	**Male**	**Female**	**Male**	**Female**	
IL-2 (pg/mL)	76.50 ± 4.5	75.60 ± 13.7	89.28 ± 2.8 #	92.47 ± 7.3 #	F (1,36) = 8.7578; p = 0.0055 (time of day) F (1,36) = 0.6578; p = 0.5719 (gender) F (1,36) = 0. 9339; p = 0.6580 (interaction)
IL-4 (pg/mL)	60.40 ± 12.1	53.6 ± 22.4	82.22 ± 6.20 #	85.7 ± 22. 9 #	F (1,36) = 14.6478; p = 0.0008 (time of day) F (1,36) = 0.0609; p = 0.8017 (gender) F (1,36) = 1.3485; p = 0.2520 (interaction)
IL-6 (pg/mL)	16. 86 ± 6.7	25.27 ± 7.3	19.21 ± 4.3	32.39 ± 11.7	F (1,36) = 0.0945; p = 0.7581 (time of day) F (1,36) = 1.4550; p = 0.2338 (gender) F (1,36) = 0.1738; p = 0.6838 (interaction)
IL-10 (pg/mL)	32.89 ± 6.2	33. 94 ± 6.6	38.06 ± 4.0	49.62 ± 6.1 *#	F (1,36) = 5.2660; p = 0.0457 (time of day) F (1,36) = 4.7577; p = 0.0401 (gender) F (1,36) = 2.1505; p = 0.1477 (interaction)
TNF-α (pg/mL)	68.36 ± 7.5	51.52 ± 7.8 *#	62.86 ± 7.0	53.54 ± 9.2	F (1,36) = 0.0145; p = 0.007 (time of day) F (1,36) = 7. 9745; p = 0.0076 (gender) F (1,36) = 0.5798; p = 0.5424 (interaction)
IFN-ϒ (pg/mL)	112.86 ± 11.4	69.70 ± 15.6 *	106.82 ± 7.3	102.3 ± 10.2 #	F (1,36) = 4.6481; p = 0.0357 (time of day) F (1,36) = 14.1099; p = 0.0009 (gender) F (1,36) = 7.7171; p = 0.0085 (interaction)
IL-17 (pg/mL)	451.38 ± 100.5	251.9 ± 84.2 *	352.55 ± 38.9	318.72 ± 47.4	F (1,36) = 0.8164; p = 0.6244 (time of day) F (1,36) = 6.7056; p = 0.0132 (gender) F (1,36) = 0.1582; p = 0.6953 (interaction)

The results represent the mean and standard error of 46 different hemodialysis patients. p-values were obtained from two-way ANOVA analyses. * comparing the male and female, considering the same collection period; # comparing the collection period (morning and afternoon), considering the same sex.

Table 5 shows the correlations of cytokines IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17 of the morning period and the afternoon period in the male and female genders. There was a positive correlation between the concentrations of cytokines IL-6, IL-10, and IFN-γ from the morning to the afternoon in the serum of women undergoing dialysis. No correlation was observed in cytokine concentrations in serum from male individuals, independently of the dialysis period.

Table 5. Pearson's correlation of cytokine levels in the morning and afternoon of men and women hemodialysis patients (n = 37).

Periods (M/V)	**IL-2 (pg/mL)**	**IL-4 (pg/mL)**	**IL-6 (pg/mL)**	**IL-10 (pg/mL)**	**TNF-α (pg/mL)**	**IFN-γ (pg/mL)**	**IL-17 (pg/mL)**
Male	r = 0.1675 p = 0.6437	r = −0.4605 p = 0.1804	r = −0.2431 p = 0.4986	r = 0.4120 p = 0.2367	r = −0.2448 p = 0.4954	r = −0.3268 p = 0.3567	r = 0.5340 p = 0.1118
Female	r = 0.4299 p = 0.2149	r = −0.1374 p = 0.7050	r = 0.6055 p = 0.0483	r = 0.6741 p = 0.0325	r = 0.2493 p = 0.4873	r = 0.6148 p = 0.0480	r = −0.3964 p = 0.2567

r = Pearson coeficiente.

4. Discussion

Hematological and biochemical changes are often seen in patients with diabetic nephropathy. One significant biochemical alteration is an increase in urea concentration, which leads to uremia due to the accumulation of pro-inflammatory cytokines resulting

from the reduced elimination of urea through the kidneys. Additionally, the dysregulation of melatonin and cortisol levels can impact the inflammatory process and kidney function [21–24]. Despite hematological alterations, anemia persists in patients with chronic kidney disease undergoing hemodialysis [25,26]. In this study, we showed that diabetic patients present alterations in the melatonin, hematological, and biochemical parameters, and cytokine levels, which are time- and gender-dependent.

The findings of this study illustrate global trends and emphasize the significance of the average age of patients needing dialysis due to chronic kidney disease. The average age of the patients was 65.4 years. In developed countries, the average age typically falls between 60 and 63 years, considerably higher than in developing countries, which ranges from 32 to 42 years [27].

Chronobiological changes can be correlated with metabolic diseases such as diabetes, which cause the breakdown of homeostasis in biological systems [12]. In this study, melatonin and cortisol levels showed differences in diabetic male and female patients undergoing hemodialysis, suggesting potential gender-dependent variations in these hormones' regulation.

Diabetic males undergoing hemodialysis show higher morning melatonin levels than females, indicating possible gender differences in melatonin secretion or metabolism. Melatonin regulates circadian rhythms and inflammation, which is crucial for hemodialysis patients [28,29]. The elevated levels in males may be linked to differences in circadian regulation or kidney function. Moreover, afternoon melatonin levels in diabetic males are lower, reflecting the natural daily decline associated with sleep–wake cycles, which can be disrupted in chronic kidney disease. This fluctuation may also be influenced by dialysis schedules and light exposure [30,31].

This study found no significant differences in cortisol levels in diabetic patients undergoing hemodialysis, suggesting that cortisol secretion can be stable or less affected by gender in this specific patient group. However, an interaction was observed between cortisol levels and other variables ($F = 3.7191$; $p = 0.0452$), indicating that overall cortisol levels were similar and could be influenced by factors such as the timing of dialysis, kidney function, or underlying inflammatory conditions [32,33].

The relationship between cortisol and these variables emphasizes the complexity of hormone regulation in diabetic patients undergoing hemodialysis. This situation highlights the necessity for further research to understand the mechanisms behind these findings. Overall, the results indicate that melatonin and cortisol levels exhibit distinct patterns influenced by gender and the time of day, which may reflect inherent physiological differences and the effects of dialysis.

The hormonal variations could affect inflammatory status, circadian rhythm disturbances, and overall health outcomes in diabetic patients undergoing hemodialysis. Diabetic nephropathy is one of the most important microvascular complications of diabetes, and its pathogenesis is complex, with metabolic changes induced by hyperglycemia and major hemodynamic changes [34,35]. In this study, the hematocrit and hemoglobin levels showed lower levels in diabetic women undergoing hemodialysis in the morning, and these values can contribute to kidney functional and structural changes.

When we analyzed hematological parameters according to gender and collection periods (morning and afternoon), we observed an increase in the hematocrit and hemoglobin levels in diabetic women who underwent hemodialysis in the afternoon, suggesting that this period can be favorable for a clinical improvement of the anemic condition for these patients. Interestingly, platelets were lower in diabetic men undergoing hemodialysis in the afternoon.

Hematological alterations with cell and platelet participation depend strongly on the conditions of the blood microflow and the local cell walls due to the different rates of

shear stress [36]. In this study, the hemorheological analysis showed that, independently of periods (morning and afternoon), the blood flow shifts showed a parabolic curve, which ascended non-linearly and was characteristic of non-Newtonian fluids.

Rheological alterations play an important role in the pathogenesis of diabetes [37], and changes in the cell and macro- and microcirculation systems vary according to their shear rates [14]. Here, the chronobiological variations of the blood flow curves were evaluated. It was observed that in the afternoon periods, there was a lower shear rate in the blood of diabetic women who underwent hemodialysis compared to women who underwent hemodialysis in the morning, suggesting that there may be greater cellular deformation in the morning period. The blood flow patterns of diabetic men undergoing hemodialysis were similar in both periods.

Diabetes can lead to changes in blood viscosity, and poor metabolic control combined with these changes can contribute to metabolic complications [12]. This study detected no difference in blood viscosity, regardless of period, in diabetic men who underwent hemodialysis. By contrast, the blood viscosity of diabetic women who underwent hemodialysis in the afternoon periods was higher than that of diabetic women who underwent hemodialysis in the morning periods. The results corroborate a previous study in which an increase in blood viscosity was observed in diabetic individuals without nephropathy [36] and indicate that hemodialysis in the afternoon periods can result in more favorable treatment outcomes for diabetic women due to the reduction in the shear rate and increased blood viscosity, providing less cellular damage.

Several factors are associated with the development and progression of diabetic nephropathy, including hyperglycemia [38]. Changes in renal function modify biochemical parameters, with increased serum levels of creatinine and urea and important deviations in the serum concentrations of hemoglobin, phosphorus, potassium, calcium, and albumin [8,39]. The study found urea levels were higher in the morning among diabetic men undergoing hemodialysis, which suggests that this period might be more harmful for these patients. Elevated urea levels can worsen the inflammatory process [25,34].

The hemodialysis intensifies the inflammatory process [40] and changes immune system components, such as complement activation, T cell activation, and macrophage function [41]. This activation of the immune system caused by tissue damage, exacerbated by the intensification of the inflammatory process, can allow the emergence and progression of diseases. The increased inflammatory response leads to the loss of peripheral tolerance to the tissues' components, which become antigenic and trigger local inflammation, as observed in chronic kidney disease [42].

In type 2 diabetes mellitus, there is an observed link between subclinical chronic inflammation and the development and progression of diabetic nephropathy. Numerous studies have shown a significant increase in inflammatory cytokines associated with the emergence and progression of chronic kidney disease [43]. Diabetes mellitus is also directly related to developing an inflammatory condition, as evidenced by increased expression of pro-inflammatory cytokines such as IL-6 and TNF-α. Research suggests that the longer the duration of the disease and/or the lack of glycemic control, the more severe the inflammatory process becomes [44,45].

In this work, the analysis of different types of cytokines in the serum of diabetic patients' hemodialysis in both periods (morning and afternoon) revealed changes in the IL-2, TNF-α, IFN-γ, IL-17, and IL-10 profile depending on the dialysis period and sex. In the morning, the cytokines TNF-α, IFN-γ, and IL-17 showed a decrease, while IL-10 increased in the afternoon period in the serum of women. These data suggest that, given the anti-inflammatory profile of IL-10 in women, the afternoon period seems more recommended for hemodialysis. It is known that IL-10 is one of the immunological mediators responsible

for reducing the inflammatory response. Its increase can be a regulatory mechanism in controlling uremia and cell activation directed by dialysis [46]. Thus, IL-10 exerts an anti-inflammatory function by inhibiting some cytokines with an inflammatory profile involved in activating immune cells [47].

The morning period's inflammatory profile for male individuals is noteworthy, with higher TNF-α, IFN-γ, and IL-17 levels. These data suggest that hemodialysis can be harmful in this period. TNF–α is known to be an important mediator of tissue damage generated by inflammation, which can be expressed and secreted in the kidneys by infiltrated macrophages and by various types of renal cells and has higher peaks of production in the morning period [48], and which corroborates with our results of the morning treatment period, where the TNF-α concentration values were higher in this phase for male individuals. Also, the levels of IFN-γ in the serum of diabetes mellitus type 2 patients with complications of nephropathy are high, which can affirm the influence of this cytokine on the progression and complication of chronic kidney disease in people with diabetes [49].

Circadian variations in clinical symptoms and concentrations of inflammatory cytokines found increased levels of both pro- and anti-inflammatory cytokines and certain hormones in sick and healthy individuals, which suggests that hormones may regulate cytokine production at specific times of the day. The pro-inflammatory cytokine TNF-α was higher in the morning in patients with polymyalgia rheumatica, a chronic autoimmune inflammatory disease most common in the elderly. Similarly, the TNF-α levels were higher in the morning for male patients undergoing treatment [49], results that are also similar to this study since the concentrations of pro-inflammatory cytokines were high in the morning dialysis period.

When cytokines were correlated, a positive correlation was observed between the concentrations of IL-6, IL-10, and IFN-γ present in the blood of female dialysis patients, both in the morning and afternoon. Still, no correlation was found in male patients. The cytokines IL-6 and IL-10 are typically involved in the inflammation process and modulation of immune response, while IFN-γ is important in defense against infections [50]. Factors such as altered immune function, differences in inflammatory responses, and disruptions of the circadian rhythm may contribute to modifications in the cytokine profile and their interactions, thus contributing to the greater immune dysfunction commonly seen in patients with chronic diseases [51–54]. These findings emphasize the complexity of immune response regulation among dialysis patients and the role of gender in the inflammatory process. Further research is necessary to understand these gender differences and their implications for treatment and patient care in dialysis populations.

In patients with chronic kidney disease undergoing hemodialysis, persistent anemia has been observed due to the influence of pro-inflammatory cytokines like IL-6 and TNF-α. These cytokines act on erythropoietic progenitor cells in a way opposite to EPO, leading to the development of anemia [55,56]. However, when analyzing hematological parameters based on gender and treatment periods, it was found that women showed an increase in hemoglobin values in the afternoon compared to the morning, suggesting that the afternoon period may be more favorable for clinical improvement in anemic conditions for this group. Also, interestingly, urea values were lower in the afternoon period in blood from diabetic women who underwent hemodialysis compared to diabetic men who underwent hemodialysis in the same period, corroborating the hypothesis that the afternoon period may be favorable for women diabetics and that this shift results in less damage to female patients.

The analysis results in this study suggest an association between different periods of hemodialysis and changes in hormones, biochemical and hemorheological parameters, and the cytokine response profile. This study's limitations include the sample size and

the presence of other common comorbidities in diabetic nephropathy that may influence hematologic, biochemical, and hormonal factors. Additional research is needed to understand better how time and gender influence patients' responses to dialysis treatment. Future studies should aim to enhance guidelines for patients undergoing dialysis therapy, as various factors, including multicenter, longitudinal, or randomized studies, are carried out to confirm our findings and minimize possible selection and confounding biases.

5. Conclusions

The findings from these studies suggest that the immunological and endocrinological profiles are more beneficial in patients who are dialyzed in the afternoon and differ between men and women. Larger studies are needed to confirm these findings and to assess whether these differences are linked to improved patient outcomes. This can be due to a higher concentration of anti-inflammatory cytokines in the afternoon, which can help reduce the inflammatory processes associated with diabetes and chronic kidney disease. Additionally, the combined data analysis indicates that hormonal, biochemical, hemorheological, and immunological parameters, such as cytokines, can be associated with chronobiological variations in patients with type 2 diabetes who are undergoing hemodialysis.

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Data Availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request and without undue reservation.

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