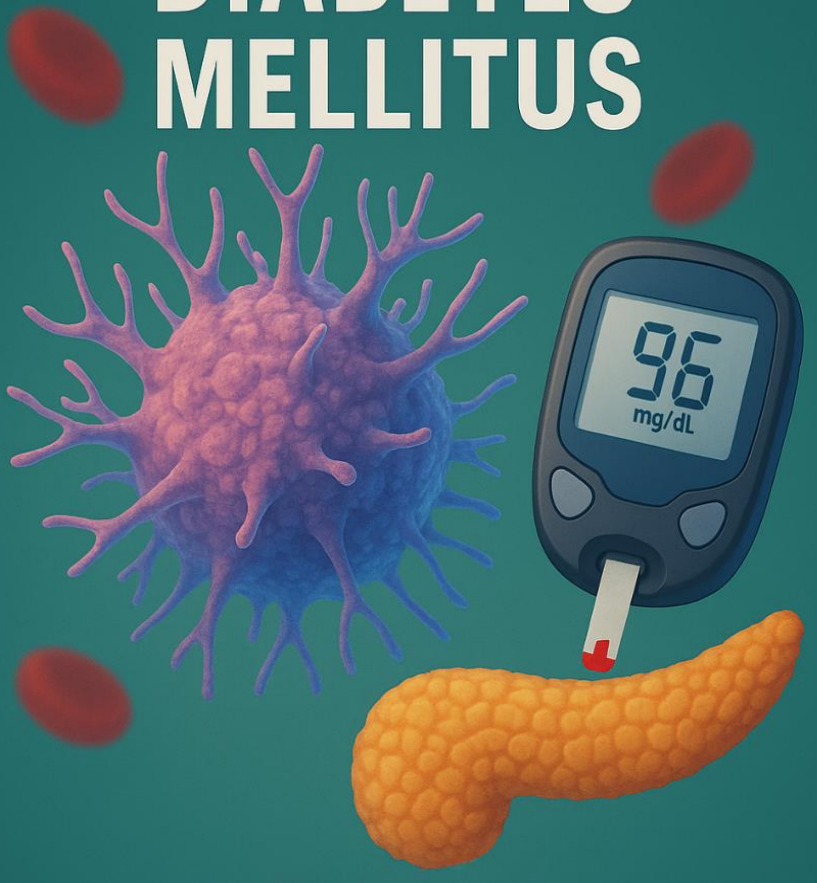
THE DENDRITIC CELL THERAPY FOR COMPLICATION OF TYPE 2 DIABETES MELLITUS



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Opening Speech

Praise be to God Almighty for the publication of this book titled *The Dendritic Cell Immunotherapy for Complication of Type 2 Diabetes Mellitus*. This book presents a highly important contribution to advancing our understanding of new approaches in the management of metabolic diseases, particularly in the context of cellular-based therapies such as dendritic cell.

As one of the world's growing health concerns, diabetes complications require innovative and comprehensive therapeutic strategies. This book highlights the concept of immunometabolism and introduces dendritic cell-based immunotherapy as a promising solution to address chronic inflammation, endothelial dysfunction, fibrosis, and pathological angiogenesis, all of which are key contributors to diabetic complications. The data presented in this book are drawn from multidisciplinary clinical trials involving experts in basic medical sciences, immunology, internal medicine, clinical pathology, and radiology. Therefore, the evidence presented is multidimensional and comprehensive, in line with the principles of *Evidence-Based Medicine*.

I believe this book will serve as an essential reference for researchers, physicians, and healthcare professionals committed to improving the quality of life of diabetes patients through more personalized and targeted therapeutic approaches. May this work contribute meaningfully to the advancement of medical science and public health, both in Indonesia and globally.

Jakarta, May 2025

Lieutenant General (Ret.) Prof. Dr. dr. Terawan Agus Putranto, Sp.Rad(K)RI

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Opening Speech

The research on "The Impact of Autologous Dendritic Cell Therapy on Albuminuria and Inflammatory Biomarkers (Interleukin-6, Interleukin-10, Tumour Necrosis Factor α) in Diabetic Kidney Disease (DKD)" is undeniably a step in the right direction in understanding and treating Diabetic Kidney Disease.

Diabetic kidney disease (DKD), is a major global health concern. A crippling illness, it often results in the need for dialysis or kidney transplantation. Albuminuria, a major clinical sign of DKD, indicate harm to the vital glomerular filtration barrier. And the development and progression of DKD are significantly influence by chronic inflammation, with key inflammatory biomarkers like interleukin-6 (IL-6), interleukin-10 (IL-10), and tumour necrosis factor α (TNF-α) connected to the intricate pathways of renal injury.

Even with current therapeutic approaches meant to slow the progression of DKD, there is still a strong need for more creative and efficient treatment approaches. Autologous dendritic cell therapy is one promising option, an immunotherapeutic strategy that modify immunological and inflammatory responses by using a patient's own immune cells. The treatment's ability to reduce inflammation and promote tissue repair has been investigated in a number of disease contexts, its use in DKD is currently a topic of increasing research interest.

The success of this project has shown the participants enduring traits: diligence, commitment, and a spirit of cooperation. We hope that those involve in the research will continue advancing medical knowledge, especially in the treatment of diabetic kidney disease, and set the groundwork for future research in this important field.

Jakarta, May 2025

Prof. Dr. dr. I Nyoman Ehrich Lister, M.Kes., AIFM., Sp.K.K.L.P.

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Foreword

Praises be to God Almighty that we have been able to complete this book, *The Dendritic Cell Therapy for Complications of Type 2 Diabetes Mellitus*. This book presents a collection of research studies focused on the potential of autologous dendritic cell therapy in addressing complications associated with type 2 diabetes mellitus.

Type 2 diabetes mellitus is known to cause both macrovascular and microvascular complications. This book concentrates specifically on microvascular complications, with a particular focus on diabetic kidney disease and diabetic neuropathy—two of the most prevalent and debilitating conditions affecting patients with type 2 diabetes. The research compiled herein aims to explore and expand the therapeutic potential of autologous dendritic cells in mitigating these complications.

We would like to extend our sincere gratitude to all contributors who shared their ideas, insights, and dedication in support of the studies featured in this book. A total of seven original research contributions are included, each authored by committed, passionate researchers. We are also deeply grateful to Prof. Dr. dr. Terawan Agus Putranto, Sp.Rad(K) RI, and Prof. I Nyoman Ehrich Lister, M.Kes, AIFM, Sp.KKLP, for their generous forewords and support throughout the journey of writing this book.

Finally, we would like to thank all individuals and institutions who lent their time, energy, and expertise in the preparation of this publication. It is our hope that this book will serve as a meaningful contribution to the scientific community and support the advancement of medical research and innovation in Indonesia and beyond.

Jakarta, May 2025

Brigadier General dr. Jonny, Sp.PD-KGH, M.Kes, MM, DCN, FISN, DABRM

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Contents

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1. Introduction

The prevalence of type 2 diabetes mellitus (T2DM) continues to rise, with more than 460 million individuals affected worldwide in 2019, and this number is projected to reach 783 million by 2045 [1,2]. In Indonesia, the prevalence of T2DM has also increased from 1.5% in 2013 to 2.0% in 2018, while the prevalence of individuals with blood glucose testing rose from 6.9% to 8.5% [3]. Currently, Indonesia ranks fourth in the world for T2DM prevalence.

Diabetic kidney disease (DKD) is a serious complication occurring in 20–50% of T2DM patients and is a major risk factor for the development of end-stage kidney disease (ESKD) [4]. DKD is characterized by persistent albuminuria, progressive decline in kidney function, and structural changes in the kidneys such as mesangial expansion and glomerulosclerosis [5]. Each year, there are approximately 2.6 million new cases of DKD globally, and this number is expected to continue to rise [6]. DKD can also occur without albuminuria, known as non-albuminuric DKD, and is often associated with macroangiopathy, interstitial fibrosis, and vascular lesions [7,8,9].

The main therapy for reducing albuminuria in DKD is the use of renin-angiotensin system inhibitors (ACE-I and ARB). The use of mineralocorticoid receptor antagonists (MRAs), such as eplerenone and spironolactone, is also applied, although they carry a risk of hyperkalemia. SGLT2 inhibitors have also shown potential in reducing albuminuria when combined with ACE-I, but the results are often not entirely satisfactory, indicating the need for further research into more effective alternative therapies [10,11].

Autologous dendritic cell (DC) transfer has been widely used for cancer, chronic infections, and autoimmune diseases, but its application in metabolic and degenerative diseases, such as diabetic kidney disease (DKD), remains unexplored [12]. Current research on cell therapy for DKD focuses on using stem cells. While animal studies indicate that stem cell therapy can improve kidney function in DKD, human trials, such as those using allogeneic mesenchymal precursor cells, have yielded limited success [13,14]. Despite the potential of stem cells in metabolic disease

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therapy, challenges such as reduced efficacy due to metabolic memory, increased cell senescence, and teratoma risk persist [15]. The current evidence supports the immunomodulatory and anti-inflammatory roles of stem cells in DKD treatment; this suggests that other cell-based products with similar mechanisms could also be viable therapeutic options [14].

Dendritic cell (DC) therapy has significant anti-inflammatory effects, as inflammation is one of the main mechanisms causing albuminuria in DKD [16]. Therefore, intervention with DC therapy may suppress inflammation and, in turn, reduce proteinuria in DKD patients. This study aims to demonstrate that DC therapy can suppress inflammation, as indicated by changes in inflammatory biomarkers (IL6, IL-10, and TNF-α), thereby reducing albuminuria in DKD patients.

2. Materials and Methods

2.1. Study Design

This study is an open-label clinical trial without randomization of subjects and without blinding of the research team. The study design is a quasi-experimental pre-test and post-test. The research procedures were conducted following applicable guidelines and regulations. This study was approved by the Ethics Committee of Gatot Soebroto Army Central Hospital (RSPAD GS) with Ethical Approval No. 72/V/KEPK/2024. All subjects provided written informed consent.

2.2. Study Subjects

The study sample consisted of DKD outpatients at the internal medicine clinic of RSPAD GS from April to May 2024. Samples were selected using non-probability consecutive sampling (quota sampling). A minimum of 68 subjects was required to detect significant changes in UACR. Sample size analysis is described in detail in Supplementary Materials S1. Subjects were recruited based on inclusion criteria: (1) male or female over 18 years old, (2) understanding and agreeing to comply with the study procedures with written consent, (3) assessed by the researcher as able to comply with the procedures, (4) judged to have overall good physical and mental health, (5) meeting the diagnostic criteria for Type 2 Diabetes Mellitus

(T2DM) as per PERKENI 2021 [17], (6) eGFR ≥ 30 mL/min/1.73 m2, (7) Urinary albumin-creatinine ratio (UACR) ≥ 30 mg/g. Patients who met any of the exclusion criteria were not included in the study. The exclusion criteria for this study are: (1) Receiving immunosuppressive treatment; (2) Presence of other kidney diseases; (3) Presence of other diseases that can cause albuminuria; (4) Presence of other types of diabetes (Type 1 diabetes, gestational diabetes, and other types of diabetes); (5) Positive pregnancy test; (6) Presence of immunodeficiency diseases such as human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV), (7) Having a condition that requires oxygen supplementation; (8) Diagnosed with invasive cancer and receiving anti-cancer therapy other than hormonal therapy for breast and prostate cancer; (9) History of thromboembolism or genetic predisposition to thromboembolism, or currently undergoing therapy with anti-thromboembolic agents other than low-dose aspirin, (10) Physical or mental disabilities that prevent normal daily activities, (11) In the researcher's assessment, the presence of medical conditions that may hinder subject participation, (12) Severe obesity (BMI > 40 kg/m2) and uncontrolled hypertension: systolic > 180 mmHg/diastolic > 100 mmHg.

Due to ethical considerations, we did not limit routine medication use, including those known to affect UACR, such as RAS inhibitors, GLP-1 Receptor Agonists, and SGLT-2 inhibitors. However, all concomitant drugs used during the study were recorded.

2.3. Study Procedure

Each subject underwent a 5-week clinical trial, including a screening phase, baseline laboratory tests, blood sampling for DC preparation, DC injection, weekly UACR evaluations for 4 weeks post-injection, and a final laboratory evaluation 4 weeks after DC injection. eGFR was measured at baseline and 4 weeks after DC injection. Inflammatory biomarker assessments (IL-6, IL-10, and TNF-α) were performed at baseline and 4 weeks after DC injection. The research protocol was approved by the Health Research Ethics Committee of RSPAD GS.

2.4. DC Preparation

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Forty cc of peripheral blood was collected. Peripheral Blood Mononuclear Cells (PBMCs) were isolated and incubated with GM-CSF (Granulocyte Macrophage Colony Stimulating Factor) and IL-4 (Interleukin-4) for 5 days to form dendritic cells. Antigen was incubated for 2 days to induce DC maturation. The cell products were injected subcutaneously into the upper arm. The exact number of autologous dendritic cells administered varies among patients, depending on individual yields. No adjustment is made to standardize the cell count across patients. The entire product, derived from 40 cc of peripheral blood, is infused, resulting in a dose that ranges between 0.5 and 8 million DCs.

2.5. Safety Evaluation

DC Injections were done by a physician. After injection, the patient was monitored for two hours for signs of immediate adverse events such as local or systemic allergic reactions, injection site inflammation, or hypersensitivity. Any adverse events that meet Common Terminology Criteria for Adverse Events (CTCAEs) v5.0 were monitored and recorded until seven days after injection.

2.6. Laboratory Testing

The Urine Albumin Creatinine Ratio (UACR) was assessed from subjects' urine. IL6, TNF-α, and IL-10 were measured from serum using sandwich-ELISA kits (Reed Biotech Ltd., Wuhan, China). eGFR was estimated from serum creatinine.

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2.7. Statistics

Median UACR levels were compared between baseline and weeks 1, 2, 3, and 4 post-intervention. Median of IL6, TNF-α, and IL-10 were compared between baseline and week 4 post-intervention. The Kolmogorov – Smirnov test was used for normality assessment. Normally distributed data were analyzed using the Paired T-test, while non-normally distributed data were analyzed using the Wilcoxon Signed-rank Test. The correlation between UACR and inflammation biomarkers was analyzed using Spearman's correlation. A multiple linear regression model was constructed to assess the relationship between the change of UACR pre-post treatment (dependent variable) and several covariates, including UACR at Baseline, BMI, cholesterol, HbA1c, eGFR, comorbidity, and medications known to affect albuminuria (ARB, ACE-I, Diuretics, SGLT-2, and GLP-1RA). This analysis aimed to evaluate the effect of these factors on changes in UACR, with all covariates entered simultaneously into the model to control for their potential confounding effects. The achieved statistical power for the linear multiple regression, based on a total sample of 69 subjects, was calculated post-hoc using G*Power version 3.1.9.7 [18]. The protocol of the calculation in G*Power is provided in Supplementary Materials S2. All statistical analyses were performed using IBM SPSS Statistics 22 (IBM, Armonk, NY, USA).

3. Results

3.1. Subject Characteristics

The study included 69 subjects who met the inclusion and exclusion criteria and completed the study procedures. Even though this sample size meets the minimum samples required for bivariate analysis of UACR change, the posthoc power analysis for multivariate analysis to control confounding factors showed a statistical power of 57.4%, although the ideal is 80%. The average age of subjects was 62 years (range: 39–83 years), with 30 men and 39 women, 36 with microalbuminuria and 33 with macroalbuminuria. Hypertension was the most common comorbidity in 65 (94.2%) subjects. The majority of subjects were overweight (35 subjects,

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50.7%). Most subjects had chronic kidney disease (CKD) stage 3b, affecting 23 (33.4%) subjects.

3.2. Changes in Albuminuria and eGFR

Each subject had UACR levels measured 5 times: once at baseline (P1) and 4 times post-DC injection (P2–P5) with weekly intervals (Table 1 and Figure 1). The median UACR levels (mg/g) at P1, P2, P3, P4, and P5 were 250, 153, 161, 125, and 164, respectively. The Wilcoxon Signed-ranks Test showed significant reductions in UACR levels from P1 to P2, P3, P4, and P5 ($p = 0.00$) compared to Baseline. Each week's comparison of UACR is described in Supplementary Materials S3, which shows that UACR levels remained stable from week to week without significant fluctuations after the initial reduction.

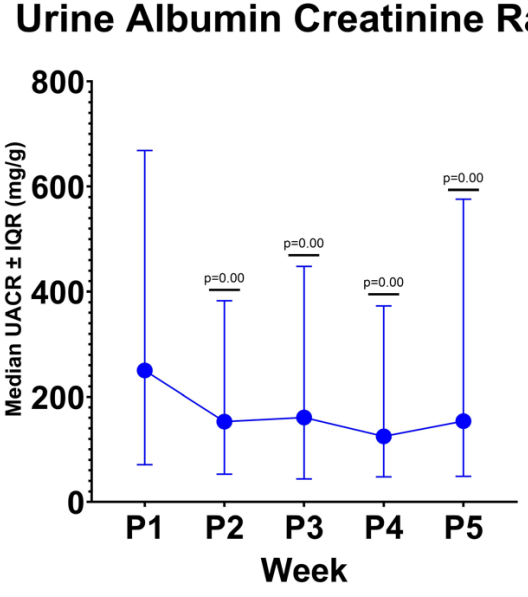

Figure 1. UACR Level by Week. UACR: urine albumin creatinine ratio, *p*:value of statistical significance, IQR: interquartile range.

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Table 1. UACR Level by Week.

Visit	UACR Median (25th–75th Percentile) (mg/g)	*p*-Value Hypothesis Test
Baseline (P1)	250 (71–668)	
Week 1 (P2)	153 (53–383)	0.00 *
Week 2 (P3)	161 (44–448)	0.00 *
Week 3 (P4)	125 (48–373)	0.00 *
Week 4 (P5)	164 (49–576)	0.00 *

* $p < 0.05$ (Significant), hypothesis test was done using Wicoxon Sign Rank in comparison to Baseline (P1).

To further evaluate kidney function, eGFR at baseline and 4 weeks post-intervention (P5) was compared. No statistically significant change was found. The median eGFR pre-immunotherapy was 60.35 mL/min/1.73 m2 (IQR 38.78–86.31), and post-immunotherapy was 56.26 mL/min/1.73 m2 (IQR 37.59–90.07 mL/min/1.73 m2); no significant changes were detected ($p = 0.478$). These results suggest that immunotherapy did not lead to a noticeable deterioration in kidney function regarding creatinine levels or eGFR following therapy. The eGFR values remained stable, indicating maintained kidney function throughout the duration of the study, which supports the safety profile of the treatment. However, the findings do not demonstrate a significant enhancement in renal function following dendritic cell therapy.

3.3. Changes in Inflammatory Biomarkers

The inflammatory biomarkers measured were IL-10 and TNF-α. IL-10 is a potent anti-inflammatory cytokine, while TNF-α is a pro-inflammatory cytokine. These markers illustrate the anti-inflammatory effects of DC therapy. This study evaluated both biomarkers at P1 and P5 (Table 2 and Figure 2). The Kolmogorov –Smirnov test indicated non-normal distribution. Thus, the Wilcoxon Signed-Rank Test was used. The median IL-6 levels (pg/mL) at P1 and P5 were 3.51 and 3.35, respectively ($p = 0.83$). The median IL-10 levels (pg/mL) at P1 and P5 were 0.74 and 0.63 ($p = 0.11$), while the median TNF-α levels at P1 and P5 were 2.16 and 1.92

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(p = 0.03). These results indicate a significant decrease in TNF-α but not IL6 and IL-10.

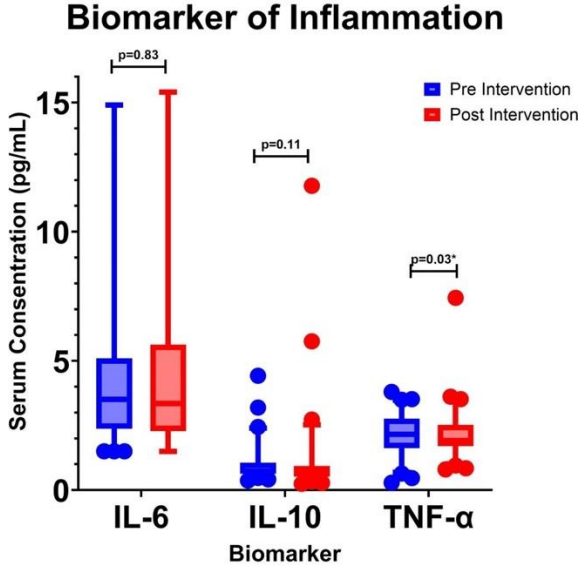

Figure 2. Concentration of Inflammatory Biomarkers. IL-10: Interleukin-10, TNF-α: Tumor Necrosis Factor-α. * p < 0.05.

Table 2. Serum Concentration of Inflammatory Biomarkers.

	Median (25th–75th Percentile) Pre Intervention/P1 (pg/mL)	Median (25th–75th Percentile) Post Intervention/P5 (pg/mL)	*p*-Value
IL-6	3.51 (2.38–5.06)	3.35 (2.34–5.27)	0.83
IL-10	0.74 (0.63–1.05)	0.63 (0.53–0.91)	0.11
TNF-α	2.16 (1.62–2.74)	1.92 (1.71–2.52)	0.03 *

* $p < 0.05$ (Statistically Significant).

3.4. Changes in Ratio of Pro-Inflammatory and Anti-Inflammatory Cytokines

The ratio of pro-inflammatory and anti-inflammatory cytokines can reflect the balance of immune responses in the body. In this study, the IL-6/IL-10 and TNF-α/IL-10 ratios were calculated (Table 3 and Figure 3). The IL-6/IL-10 ratio in P1 and P5 was 4.68 and 4.79 (p = 0.063), indicating no

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significant change. Similarly, for the TNF-α/IL-10 ratio, there was no significant difference between P1 (2.94) and P5 (3.16) with a p-value of 0.115.

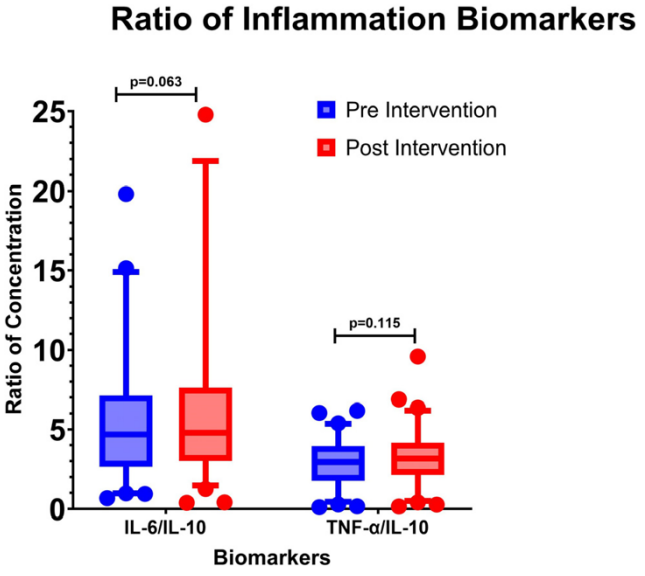

Figure 3. Ratio of pro-inflammatory and anti-inflammatory cytokines in response to treatment. IL-10: Interleukin-10, TNF-α: Tumor Necrosis Factor-α

Table 3. Ratio of Pro-inflammatory and anti-inflammatory cytokines.

	Median (25th–75th Percentile) Pre Intervention (pg/mL)	Median (25th–75th Percentile) Post Intervention (pg/mL)	*p*-Value
Ratio IL-6/IL-10	4.68 (2.66–7.04)	4.79 (3.03–7.58)	0.063
Ratio TNF-α/IL-10	2.94(1.77–3.85)	3.16(2.21–4.12)	0.115

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3.5. Correlation of UACR and Inflammation Biomarkers

To examine the interaction between each inflammatory biomarker and UACR post-intervention, a Spearman correlation test was conducted on P5 (Table 4 and Figure 4). Based on the results of this analysis, there was a significant negative correlation between UACR and IL-10, with a correlation coefficient of −0.281 (p = 0.019). This indicates that an increase in IL-10 levels tends to be followed by a decrease in UACR. Additionally, there was a significant positive correlation between UACR and TNF-α, with a correlation coefficient of 0.312 (p = 0.009). This suggests that an increase in TNF-α levels tends to be followed by an increase in UACR. No significant relationship was found between UACR and IL-6 (correlation coefficient = 0.172, p = 0.157). Similarly, the correlations between IL-10 and TNF (correlation coefficient = -0.004, p = 0.975), as well as between IL-10 and IL-6 (correlation coefficient = 0.141, p = 0.249), were not significant. The correlation between TNF and IL-6 was also not significant (correlation coefficient = 0.179, p = 0.141).

Table 4. Spearman Correlation Inflammation Biomarkers and UACR Post Intervention.

Correlation Coefficient (*p* Value)	IL-6	IL-10	TNF
UACR	0.172 (0.157)	−0.281 (0.019) *	0.312 (0.009) *
IL-6	-	0.141 (0.249)	0.179 (0.141)
IL-10	-	-	−0.004 (0.975)

* $p < 0.05$ (Statistically Significant).

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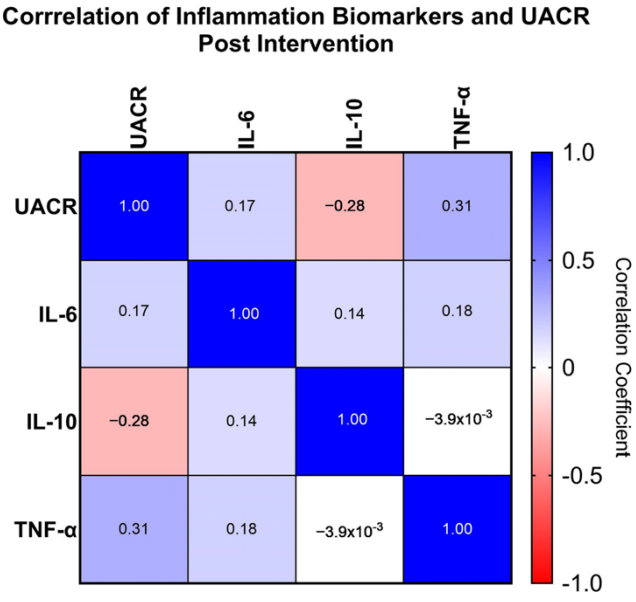

Figure 4. Heatmap of Correlation of UACR and Inflammation Biomarker. IL-10: Interleukin-10, TNF-α: Tumor Necrosis Factor-α.

3.6. Multivariate Analysis

A multiple linear regression analysis assessed the factors associated with changes in UACR pre and post intervention (ΔUACR) (Table 5 and Figure 5). The model included UACR_Baseline, HbA1c, Medications, BMI, Comorbidities, eGFR, and cholesterol as predictors. The model explained 55.0% of the variance in ΔUACR (R2 =0.550), with an adjusted R2 of 0.498, indicating good explanatory power. The overall model was statistically significant ($F(7,61) = 10.635$, $p< 0.001$), suggesting that the predictors collectively have a significant impact on ΔUACR. Among the predictors, UACR_at baseline ($B = −0.159$, $p < 0.001$) and cholesterol ($B = −1.375$, $p = 0.040$) were significantly associated with ΔUACR. Higher baseline UACR levels and cholesterol were associated with a decrease in ΔUACR. The other variables, including HbA1c ($B = −22.984$, $p = 0.135$), medications ($B = 6.154$, $p = 0.936$), BMI ($B = −1.868$, $p = 0.761$), eGFR ($B = −0.069$, $p = 0.950$), and comorbidities ($B = −50.136$, $p = 0.527$), did not show significant associations with ΔUACR. Residual analysis revealed a mean residual of 0.00 with a standard deviation of 194.03,

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indicating substantial unexplained variability in ΔUACR. Standardized residuals ranged from −3.656 to 2.935, suggesting the presence of potential outliers. Despite these outliers, the distribution of residuals was generally well-centered around zero, indicating an overall unbiased fit of the model.

Table 5. Multiple Linear Regression Factors Associated with Changes in UACR (ΔUACR).

Predictor [1]	Unstandardized Coefficient (B)	Std. Error	*p*-Value
(Constant)	508.293	218.750	0.023
Medications	6.154	75.899	0.936
BMI	−1.868	6.116	0.761
Cholesterol	−1.375	0.654	0.040 *
eGFR	−0.069	−0.069	0.950
Comorbidities	−50.136	78.775	0.527
HbA1c	−22.984	15.177	0.135
Baseline UACR	−0.159	0.028	<0.001 *

* $p < 0.05$ (significance); [1] Dependent Variable: ΔUACR was calculated from [UACR at Week 4] − [UACR at Baseline].

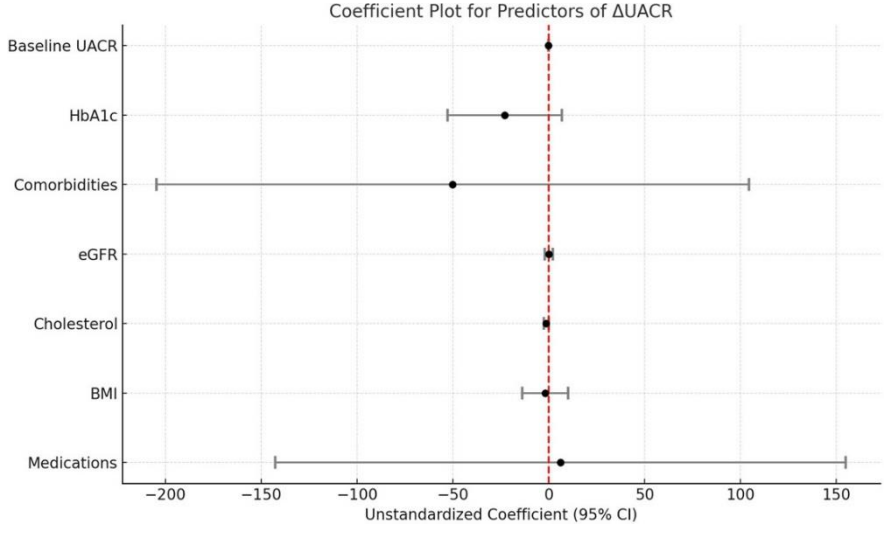

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Figure 5. Coefficient Correlation of Multiple Linear Regression of Prediting ΔUACR. eGFR: Estimated Glomerular Filtration Rate, BMI: Body Mass Index, UACR: Urine Albumine Creatinine Ratio, HbA1c: Glycated Hemoglobin.

In summary, the results suggest that UACR at baseline and cholesterol are the primary factors influencing changes in UACR, while the other variables showed no significant effects. The model's unexplained variance suggests that additional factors may need to be considered to enhance the predictive accuracy.

4. Discussion

Most of the comorbidities found in the subjects were hypertension. This finding aligns with the study by Soeatmadji et al., which concluded that patients with T2DM in Indonesia predominantly have hypertension as a comorbidity, amounting to 43% [19]. Furthermore, Ismail et al. concluded that individuals in the pre-hypertension and hypertension stages have a higher risk of developing T2DM, with respective RR values of 1.39, 95% CI 1.14–1.69, and RR 1.75, 95% CI 1.43–2.16 [20]. Hypertension and DKD have a complex relationship with interrelated pathophysiological mechanisms. Hypertension not only worsens kidney damage but also arises as a result of renal dysfunction in diabetic patients. In diabetes, increased vascular resistance due to vascular remodeling leads to hypertension. Arteriolar remodeling in DKD raises glomerular pressure, further exacerbating kidney damage and worsening hypertension [21]. This condition is worsened by hyperinsulinemia and fluid overload, which ultimately increase systemic blood pressure [22]. This can explain the findings of this study, where over 90% of diabetic patients with albuminuria—a marker of kidney damage—have hypertension as a comorbid condition.

Overall, there was a significant decrease in UACR from baseline to post-intervention with DC. The median UACR decreased from baseline to the first week, indicating a response to the intervention. A slight increase occurred in the second week, but it decreased again in the third week.

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Subsequently, a slight increase was observed in the fourth week. However, the median UACR levels from the first to the fourth week after the intervention remained lower than the baseline and were statistically significant. This indicates that DC can reduce UACR in DKD patients for up to four weeks post-intervention.

To date, no studies have examined the effects of reducing UACR in DKD patients following DC administration. Therefore, the reduction in UACR shown in this study can be compared with currently available standard treatments. Telmisartan, an Angiotensin-II Receptor Antagonist (ARB), has been shown to be effective in reducing UACR, with a clinical trial finding that 52% of subjects experienced a 39.6% reduction in UACR from baseline [23]. Enalapril (an ACE inhibitor) can reduce UACR by 65.7% after one year of regular use compared to placebo [24]. GLP-1 Receptor Agonists (GLP-1 RA) have also been shown to significantly reduce UACR compared to placebo, with reductions ranging from 17% to 33% [25]. SGLT2 inhibitors, such as dapagliflozin, can also reduce UACR by 19% to 22% compared to placebo [26]. In this study, the analysis of all subjects revealed that DC administration could reduce UACR by 34.4–50% up to 4 weeks after injection.

IL-10 levels did not change significantly after the intervention. IL-10 is a potent anti-inflammatory and immunosuppressive cytokine produced by macrophages, Th2 cells, DC, B cells, monocytes, neutrophils, eosinophils, and mast cells [27]. T2DM patients have lower IL-10 levels [28]. This is further complicated by immune cell resistance to the anti-inflammatory effects of IL-10 [29]. IL-10 directly reduces glomerular macrophages' recruitment, activation, and proliferation in vivo. IL-10 significantly reduces macrophage-mediated glomerular injury and improves albuminuria conditions [30].

No significant eGFR change was found after the intervention. Conventional treatment for DKD also found limited effects on eGFR despite long-term treatment [23,24,25,26]. However, some medications are found to protect declining kidney function in diabetic patients. To improve eGFR, a major recovery of kidney structure should occur. Nephron regeneration in chronic kidney disease requires at least twelve

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weeks, so longitudinal monitoring of eGFR at least every three months should be done [31]. Hence the effect of DC immunotherapy on eGFR remains inconclusive.

This study also found a decrease in UACR levels after DC intervention and a reduction in TNF-α levels. This finding is consistent with previous research showing a positive correlation between TNF-α levels and albuminuria. Several studies have indicated that TNF-α receptors released into circulation can serve as markers of declining renal function in patients with type 2 diabetes mellitus [32,33]. A study found a direct correlation between TNF-α levels and UACR in T2DM patients [34]. A significant increase in TNF-α was also found in DKD patients [35,36]. Multivariate analysis revealed an independent relationship between urinary TNF-α and albuminuria. Furthermore, TNF receptor levels are associated with worsening renal function, even in individuals with normoalbuminuria [37]. Therefore, the decrease in UACR levels following DC administration observed in this study is mediated by a reduction in TNF-α levels.

Pro-inflammatory cytokines play a crucial role in fighting pathogens, while anti-inflammatory cytokines function to prevent tissue damage caused by an excessive immune response. An imbalance in this system can lead to issues: excessive pro-inflammatory conditions can trigger allergies and autoimmune diseases, whereas excessive anti-inflammatory responses can result in immunosuppression [38]. In individuals with DKD, there is often a tendency towards pro-inflammatory overactivation, which can lead to tissue damage [39]. In this study, the results showed no increase in the pro-inflammatory/anti-inflammatory ratio, indicating that the intervention did not alter the immune response balance. Median analysis revealed a significant decrease in TNF-α levels but no change in the TNF-α/IL-10 ratio. This suggests that the reduction in TNF-α was balanced by changes in IL-10, thus posing no risk of immunosuppression.

A Spearman correlation analysis was conducted post-intervention to examine the relationship between the tested parameters. The results indicated that IL-10 and TNF-α after therapy significantly correlated with UACR, while IL-6 did not significantly correlate with UACR or other biomarkers. These findings are consistent with previous studies, where IL-

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10 was negatively correlated with UACR levels, whereas TNF-α was positively correlated with UACR [40]. Thus, this analysis further supports the conclusion that the reduction in UACR observed post-intervention is mediated by a decrease in TNF-α.

This study has several limitations, including not restricting the use of antihypertensive and antidiabetic medications known to lower UACR, such as RAS inhibitors, GLP-1 Receptor Agonists, and SGLT-2 inhibitors. The short duration of the study (only 4 weeks) limits the evaluation of the long-term impact of the intervention, considering that albuminuria in DKD is progressive. Additionally, the intervention was only performed once, warranting further exploration regarding the optimal frequency and duration to achieve more significant and sustainable therapeutic benefits. Further research is needed to improve medication control, explore the specific effects of DC administration, and determine the most effective dosing and intervention schedule.

Multivariate analysis was conducted to measure the influence of confounding factors on the change of UACR in this study. Cholesterol and baseline UACR contribute significantly to the variance of ΔUACR. Other variables (e.g., BMI, HbA1c, eGFR, complications, and drug use) do not significantly impact the change in UACR, indicating that they may not play a major role in this outcome within the context of this model. Cholesterol is a known predictor of metabolic disorders, and it is known to positively correlate with UACR [41]. Baseline UACR signifies the progression of kidney damage; advanced damage influences the efficacy of this treatment. DC immunotherapy has lower efficacy in people with higher cholesterol and people with more advanced kidney damage. However, due to the limitation of sample size, this study has not reached adequate statistical power (Supplementary Materials S1) to confidently conclude that other variables do not influence UACR. So, follow-up studies involving more subjects should be conducted.

This study was not designed as double-blind due to ethical considerations. In future research, increasing the clinical significance will require patient stratification based on various criteria, such as comorbidity, medication use, degree of kidney damage, vascular remodeling, and glycemic control.

A successive clinical trial with a gradually larger cohort that eventually led to a double-blind study design should be conducted.

Another limitation of this study is that dose standardization was not implemented. This is due to a couple of reasons. Firstly, this research did not aim to assess the dose-response relationship; rather, it aimed to determine the effect of dendritic cell (DC) therapy on albuminuria and elucidate its underlying mechanism through inflammatory biomarkers. Additionally, due to the personalized nature of this intervention, the number of autologous DCs administered was based on individual yields, allowing for a tailored therapeutic approach. Nevertheless, standardized dosing is essential for accurately evaluating the dose-response relationship and should be considered in follow-up and future studies. Regardless of the limitations, the significant finding of this study can be the justification for further study.

5. Conclusions

This study demonstrates that DC administration can significantly reduce UACR levels in DKD patients for up to four weeks post-intervention compared to baseline, with stabilization of levels in subsequent weeks and deterioration in eGFR. This indicates that the intervention effect was limited to reducing albuminuria without promoting regeneration of glomerular filtration capacity. Additionally, there was a significant reduction in TNF-α levels, supporting the theory that inflammation plays a key role in the pathophysiology of DKD, while IL-10 levels did not change significantly. This suggests a possible anti-inflammatory mechanism of DC administration associated with suppressing the pro-inflammatory cytokine TNF-α rather than promoting the anti-inflammatory cytokine IL-10. Given the low statistical power, further studies with larger samples are recommended to confirm and further investigate the factors associated with treatment response.

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The Effect of Autologous Dendritic Cell Immunotherapy on Kidney Function and Endothelial Dysfunction of Patients with Diabetic Kidney Disease (DKD): An Open Label Clinical Trial

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1. Introduction

DKD is a clinical syndrome characterized by pathological features such as persistent albuminuria, progressive decline in renal function, and histological changes in the kidneys [1]. Structural and functional changes include glomerular mesangial expansion, basement membrane thickening, podocyte loss, nodular glomerulosclerosis, and endothelial cell damage. In the early stages, tubular hypertrophy occurs, which later progresses to interstitial fibrosis with tubular atrophy [2].

One of the key pathological features of DKD is endothelial damage in the glomerular basement membrane, which leads to impaired kidney filtration, resulting in albuminuria [3]. Endothelial dysfunction in DKD is closely related to persistent hyperglycemia, a hallmark of diabetes. High blood glucose levels lead to the formation of advanced glycation end products (AGEs) which, through interaction with the AGE receptor (RAGE), activate various inflammatory pathways [4,5,6,7]. This process mediates the development of a low-grade chronic inflammatory condition in diabetes [8]. This complex interaction involves multiple biochemical and molecular pathways, with chronic inflammation acting as a major driver in disrupting endothelial homeostasis and contributing to the progression of DKD [9]. Chronic inflammation triggers the formation of reactive oxygen species (ROS), which cause oxidative stress. Oxidative stress disrupts the synthesis and availability of nitric oxide, a crucial molecule for endothelial function responsible for vasodilation, maintaining blood flow, and inhibiting platelet aggregation [10].

Another key aspect of inflammation-induced endothelial dysfunction in DKD is activating the nuclear factor kappa-light-chain-enhancer (NF-κB) pathway. This pathway is central to the inflammatory response, and its activation in endothelial cells increases the expression of various adhesion molecules such as ICAM (intercellular adhesion molecule) and VCAM (vascular cell adhesion molecule). The elevated expression of these molecules facilitates leukocyte adhesion and migration to the endothelium, further exacerbating endothelial damage [11]. VEGF (vascular endothelial growth factor) is upregulated as a compensatory response to endothelial damage. VEGF plays a crucial role in

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angiogenesis, endothelial cell proliferation and survival, and tissue regeneration in the kidneys. Therefore, in the early stages of kidney damage, VEGF levels increase. However, in severe kidney damage, VEGF levels decrease due to extensive damage where kidney cells can no longer express VEGF [12].

The use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) has been proven to reduce proteinuria and slow the decline in kidney function. Recent studies have highlighted the impact of new pharmacological agents, such as sodium-glucose cotransporter 2 (SGLT2) inhibitors, on the management of diabetic kidney disease (DKD). Empagliflozin, an SGLT2 inhibitor, has demonstrated significant renal and cardiovascular benefits in patients with type 2 diabetes and pre-existing kidney disease [13]. However, the efficacy of these drugs is generally unsatisfactory. Furthermore, ACE inhibitors, can cause various side effects such as hyperkalemia, angioedema, and decreased renal function that can be life threatening [14,15,16]. Therefore, it is essential to explore alternative approaches that can enhance the effectiveness of current treatments while minimizing their side effects.

One promising avenue lies in targeting the immune system, which plays a central role in the progression of DKD and other chronic inflammatory conditions. In this context, dendritic cells (DCs) have garnered significant attention due to their pivotal role in bridging innate and adaptive immunity. DCs are unique in their ability to process and present antigens to other immune cells, which is vital in regulating immune responses [17]. In inflammatory conditions, DCs play a dual role. They can trigger immune responses to fight infection and cause inflammation, but they also have the ability to control excessive inflammatory responses. This is achieved by inducing regulatory T cells (Tregs) and producing anti-inflammatory cytokines. Thus, DCs are crucial in maintaining immune balance and preventing autoimmune diseases as well as excessive inflammatory responses that can damage body tissues [18].

DCs can be generated outside the body by isolating peripheral blood monocytes and culturing them in differentiation media containing

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granulocyte–macrophage-stimulating factor (GM-CSF) and interleukin-4 (IL-4) [19]. These ex vivo-generated DCs are then transferred back to the patient's body, where they efficiently migrate to the lymph nodes and perform their function in vivo. Autologous DC transfer can potentially improve and prevent disease progression in DKD patients [20]. However, to date, no studies have investigated the effect of autologous DC administration on clinical outcomes in DKD. Given the critical role of endothelial dysfunction in the pathogenesis of DKD and the role of DCs in maintaining endothelial function, further studies are needed to assess the effect of DC immunotherapy on endothelial dysfunction. Therefore, this study investigated the effect of DC immunotherapy on endothelial dysfunction in DKD patients.

2. Materials and Methods

2.1. Study Design

This study is an open-label clinical trial without randomization and without the use of blinding. The study design is quasi-experimental with a pretest and posttest approach. Sham–control group, blinding, and placebo are not utilized due to ethical considerations. The research procedures were developed in accordance with applicable guidelines and regulations, ensuring subject protection and ethical compliance. This study has received approval from the Ethics Committee of Gatot Soebroto Army Central Hospital (RSPAD GS) with Ethical Clearance Number 108/VIII/KEPK/2024. All subjects provided written informed consent before participating.

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2.2. Study Subjects

The research subjects were DKD patients receiving outpatient care at the internal medicine clinic of RSPAD GS from April to May 2024. Sampling was conducted using a nonprobability sampling method, specifically consecutive sampling (quota sampling). Based on the calculations, a minimum of 67 subjects were required to detect significant changes in the urine albumin–creatinine ratio (UACR). The subjects included in this study (inclusion criteria) were diabetic patients fulfilling criteria from the Indonesian Endocrinology Association (PERKENI) 2021 guidelines, aged over 18 years, with an eGFR of ≥30 mL/min/1.73 m2 and a urinary albumin–creatinine ratio (UACR) of ≥30 mg/g. However, the study excluded patients known to be receiving immunosuppressive treatment, anticancer therapy (except hormonal therapy), or antithrombotic treatment other than low-dose aspirin. Patients with other kidney diseases, other forms of diabetes, cancer, immunodeficiency diseases, or those receiving oxygen supplementation therapy were also excluded from the study.

The sample size was determined using sample size formula for comparing means in numerical data for a single population.

$$n = \left(\frac{[Z_\alpha + Z_\beta]S}{x_1 - x_2} \right)^2 \quad (1)$$

n : Sample Size
S : Standard Deviation
x1 − x2 : Effect Size
Zα : Z-score with alpha 0.05 = 1.64
Z : Z-score with beta (power) 0.8 = 0.84

Based on a previous study by Kashiwagi et al., the standard deviation (σ) of UACR in patients with T2DM is 497.8 mg/g [21]. Using a confidence level of α = 0.05, a power of 0.8, and an expected change in UACR of 150 mg/g, the minimum sample size for this study was 68 subjects. Post hoc statistical power calculation for the multivariate model is described in Section 2.4.

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2.3. Study Procedure

The study lasted for five weeks and began with a screening phase, where the subjects' conditions were evaluated to ensure they met the inclusion criteria. After passing the screening, subjects underwent laboratory tests to collect baseline data, including clinical parameters, and a blood draw of 40 cc to generate dendritic cells (DCs). DC and antigens were prepared as described in previous studies [22,23]. To summarize, the procedure was as follows: The peripheral blood was processed to isolate peripheral blood mononuclear cells (PBMCs) based on density gradient medium using LymphoprepTM (StemcellTM Technologies Inc., Vancouver, BC, Canada), which were then incubated with MoDC differentiation media supplemented with granulocyte macrophage colony stimulating factor and interleukin-4 (Aivita Biomedical, Irvine, CA, USA) for five days in 37 °C with 5% CO2. Subsequently, maturation was initiated by incubating the DCs with antigens for two days (Aivita Biomedical, Irvine, CA, USA). The cell products were injected subcutaneously by a trained physician. Subjects were closely monitored for 30 min post administration to observe for any signs of allergic reactions. Any adverse events were documented for up to seven days following the injections. Moreover, all subjects continued their routine therapy without any modifications throughout the study period.

Following the DC administration, subjects' conditions were monitored through weekly evaluations of the urine albumin–creatinine ratio (UACR) over a four-week period. Further laboratory evaluations were conducted in the fourth week after injection, including measuring endothelial dysfunction biomarkers (ICAM, VCAM, and VEGF) and Creatinine to monitor the estimated glomerular filtration rate (eGFR). The Urine Albumin Creatinine Ratio was assessed using the turbidimetric method, while ICAM, VCAM, and VEGF were measured from serum using a sandwich-ELISA kit (Reed Biotech Ltd., Wuhan, China). All stages of this study were carried out in accordance with the protocol approved by the Health Research Ethics Committee of RSPAD GS (No. 108/VIII/KEPK/2024), ensuring compliance with ethical standards and subject safety.

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2.4. Statistics

The median UACR at weeks 1, 2, 3, and 4 post intervention was compared to baseline. The median eGFR at week 4 post intervention was also compared to baseline. Endothelial dysfunction biomarkers at week 4 post intervention were compared to baseline. Normality testing of the data was conducted using the Shapiro–Wilk test. Hypothesis testing for normally distributed data was performed using the paired t-test, while non-normally distributed data were analyzed using the Wilcoxon sign rank test. Spearman correlation analysis was then conducted for each parameter at baseline and week 4 post intervention. A multivariate analysis was performed to account for the effects of confounding factors, which in this study included age, baseline UACR, HbA1c, eGFR, and concomitant drug use. By the end of the study, the total sample size was 69 subjects. Post hoc power analysis for the multivariate model was conducted using G-power [24]. With a medium effect size of (0.15), a significance level of $\alpha = 0.05$, 5 predictors, and a sample size of 69, the resulting statistical power ($1-\beta$) was 0.65. However, this is below the generally accepted threshold of 0.80 for adequate power. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using SPSS version 13 (IBM, Armonk, NY, USA), and data visualization was conducted using Graphpad Prism 8 (Graphpad Software, LLC, San Diego, CA, USA).

3. Results

3.1. Subject Characteristics

A total of 69 subjects completed the study and were analyzed. The average age of the subjects was 62 years, with an age range of 39 to 83 years. The gender ratio of the subjects (male:female) was 1:1.3. The majority of subjects were of Javanese ethnicity (42%), followed by Chinese (13%), Betawi (12%), Batak (12%), Sundanese (7%), Minangkabau (6%), and others (9%). A total of 94.2% of the subjects had hypertension, with most receiving ACE inhibitor therapy (63.8%). The majority of subjects were also on insulin therapy (63.8%). Based on body mass index (BMI), most of the subjects were categorized as overweight (50.7%), with median triglyceride levels of [145 (100–187) mg/dL], LDL [115 (93–158) mg/dL],

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and HDL [47 (41–53) mg/dL]. A total of 52.2% of the subjects had a UACR level < 300 mg/g (microalbuminuria), while 47.8% had a UACR level > 300 mg/g (macroalbuminuria). Based on eGFR criteria, most subjects had an eGFR of 30–44 mL/min/1.73 m2 (33.4%) and 60–89 mL/min/1.73 m2 (30.4%). A complete description of subject characteristics can be seen in Supplementary Table S1.

No serious adverse events were observed in this study. Adverse events were limited to local reactions such as pain and swelling at the injection site.

3.2. Clinical Outcome

The change in median UACR (mg/g) in patients with diabetic kidney disease (DKD) following autologous dendritic cell immunotherapy is described in Figure 1. At baseline, the median UACR was 250 mg/g (IQR 71–668 mg/g). After therapy, a significant reduction compared to baseline UACR was observed at week one (153 mg/g; IQR 53–383 mg/g), week two (161 mg/g; IQR 44–448 mg/g), week three (125 mg/g; IQR 48–373 mg/g), and week four (164 mg/g; IQR 49–576 mg/g) compared to baseline, with all p-values < 0.05 (Wilcoxon sign rank test). Each week comparison shows that the reduction is sustained up to four weeks without any significant fluctuation (week 1 vs. week: $p = 0.827$; week 2 vs. week 3: $p = 0.0263$; week 3 vs. week 4: $p = 0.623$). This significant decrease in UACR indicates an improvement in kidney function related to albuminuria following immunotherapy.

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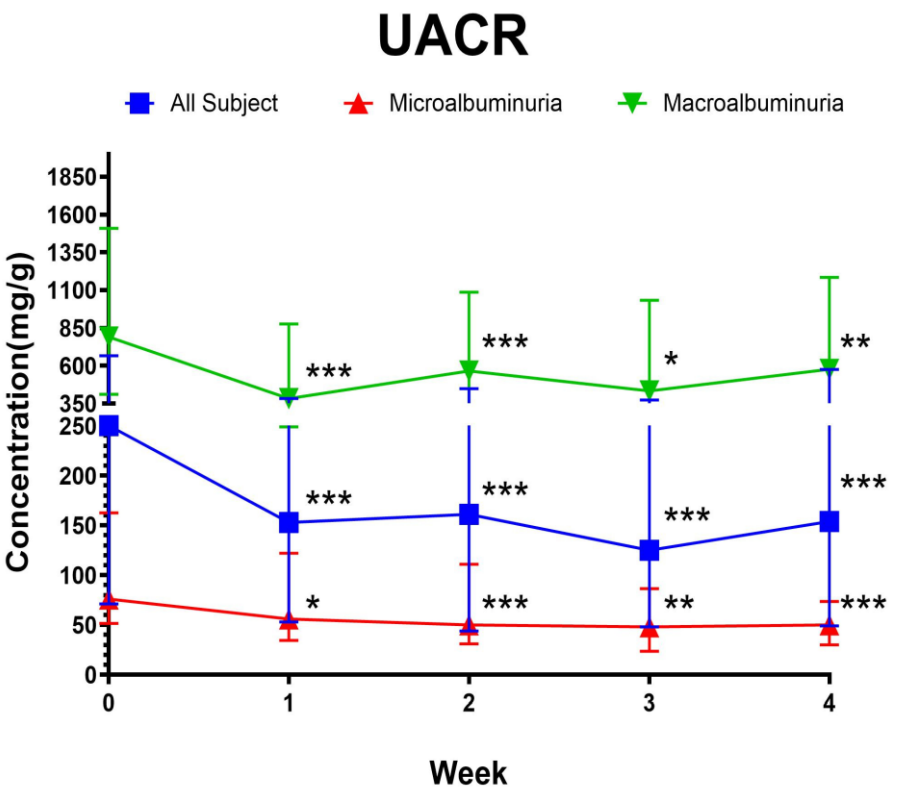

Figure 1. Weekly comparison of urine albumine–creatinine ratio (UACR). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; p-value was calculated in comparison to baseline (week 0) using the Wilcoxon sign rank test. Data were presented asa median ± interquartile range (IQR). All subjects n = 69, microalbuminuria n = 36, macroalbuminuria n = 36.

Subjects were categorized into two subgroups based on their baseline albuminuria levels: microalbuminuria (UACR 30–300 mg/g) and macroalbuminuria (UACR > 300 mg/g). Participants in the microalbuminuria subgroup showed a significant reduction in UACR levels from baseline (76 mg/g; IQR 51.50–162.50 mg/g) to week one (56 mg/g; IQR 34.50–122.00 mg/g), week two (50 mg/g; IQR 31.00–111.00 mg/g), week three (48 mg/g; IQR 23.50–86.50 mg/g), and week four (50 mg/g; IQR 30.00–73.50 mg/g), with all reductions achieving statistical significance ($p < 0.05$, Wilcoxon signed rank test). Similarly, participants in the macroalbuminuria subgroup exhibited a significant reduction in

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UACR levels from baseline (792 mg/g; IQR 412.00–1508.00 mg/g) to week one (383 mg/g; IQR 249.00–878.00 mg/g), week two (566 mg/g; IQR 281.00–1086.00 mg/g), week three (434 mg/g; IQR 275.00–1031.00 mg/g), and week four (577 mg/g; IQR 296.00–1183.00 mg/g), all with $p < 0.05$ (Wilcoxon signed rank test). Weekly comparisons indicated that the reductions in UACR levels were sustained over the four-week period without significant fluctuations ($p > 0.05$). Additionally, the percentage reduction in UACR from baseline to week four was calculated, showing an overall reduction of 25.41% ± 36.40% (mean ± standard deviation) across all subjects. In the microalbuminuria subgroup, UACR decreased by 32.12% ± 39.05%, while the macroalbuminuria subgroup demonstrated a reduction of 18.09% ± 32.85%. However, the difference in percentage reduction between the two subgroups was not statistically significant ($p = 0.113$, independent t-test).

Table 1 presents the results of the median creatinine levels and estimated glomerular filtration rate (eGFR) analysis before and after autologous dendritic cell immunotherapy in patients with diabetic kidney disease (DKD). No significant changes to either creatinine or eGFR were detected ($p > 0.05$). These results suggest that immunotherapy did not lead to a noticeable deterioration nor improve the kidney function in terms of creatinine levels or eGFR following therapy.

Table 1. Creatinine and estimated glomerular filtration rate levels.

Parameter	Pre Intervention	Post Intervention	*p*-Value
Creatinine (mg/dL)	1.04 (0.82–1.62)	1.12 (0.78–1.66)	0.467
eGFR (mL/min/1.73 m^2)	60.35 (38.78–86.31)	56.26 (37.59–90.07)	0.478

Hypothesis testing was conducted using the Wilcoxon sign rank for all parameters. Data were presented as median (IQR).

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3.3. Predictors of UACR Change

Multivariate analysis using multiple linear regression was conducted for potential confounding factors (Figure 2). Concomitant drug use was defined as medication that affect UACR such as RAAS inhibitors, diuretics, and SGLT-2. ΔUACR was calculated as the difference between UACR at week 4 and UACR at baseline. The results of the multiple linear regression analysis, with ΔUACR as the dependent variable, are presented in Figure 2. The model revealed that baseline UACR ($B = -0.185$, $p < 0.001$) and HbA1c ($B = -33.270$, $p = 0.021$) were significant predictors of ΔUACR. Baseline UACR demonstrated a strong negative association with changes in UACR, indicating that higher baseline UACR values were associated with a greater reduction in UACR. Similarly, HbA1c showed a significant negative relationship, suggesting that poorer glycemic control was associated with a smaller improvement in UACR. In contrast, the variables concomitant drugs ($B = 9.839$, $p = 0.903$), age ($B = 0.133$, $p = 0.966$), and eGFR ($B = -0.487$, $p = 0.661$) did not show statistically significant associations with ΔUACR. These findings highlight the critical role of baseline UACR and glycemic control (HbA1c) in influencing changes in albuminuria, and thus, should be taken into consideration.

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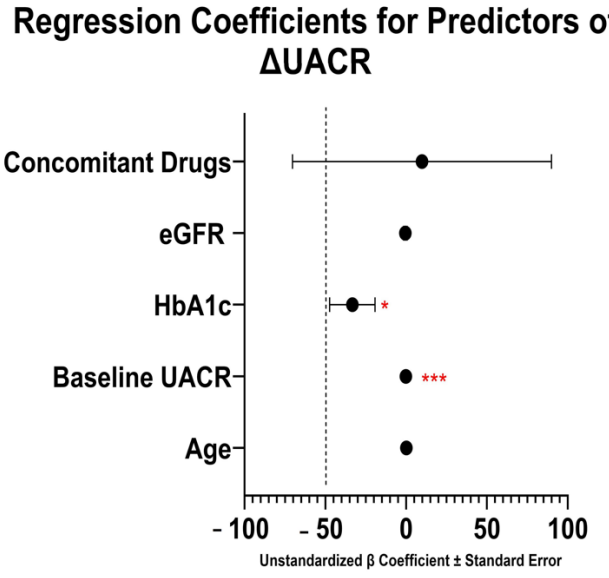

Figure 2. Regression coefficients with standard errors for predictors of ΔUACR. ΔUACR was calculated from the difference between UACR at week four and baseline. Independent variables significantly predict ΔUACR [$F(5, 63) = 12.97$; $p < 0.001$]. This model explains 50.7% of variance in ΔUACR ($R2 = 0.507$). Constant ($\beta 0$) in this model is 292.27 ± 255.90. * $p < 0.05$, *** $p < 0.001$, eGFR: estimated glomerular filtration rate, HbA1c: A1c hemoglobin, UACR: urine albumin–creatinine ratio.

3.4. Endothelial Dysfunction Biomarkers

To reveal the mechanism of action in reducing UACR, endothelial dysfunction biomarker levels were measured before and after intervention (Table 2). The levels of ICAM, VCAM, and VEGF did not significantly change after intervention ($p > 0.05$). However, based on analysis of change in UACR predictors after intervention, baseline UACR and Hb1c levels were identified as the most significant predictors. To analyze whether endothelial dysfunction plays role in how glycemic control and baseline UACR predict response to treatment, sub-group analysis of the endothelial dysfunction biomarker was conducted based on those categories. No significant differences were found in ICAM and VEGF based on any sub-group. However, subjects with good glycemic control showed a significant increase in VCAM after intervention ($p = 0.048$), with 1326.01 ng/mL

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(IQR 990.92–1522.84 ng/mL) at baseline and 1391.10 ng/mL (IQR 1077.64–1600.94 ng/mL) at four weeks after intervention (Figure 3). On the contrary, subjects with macroalbuminuria showed a significant decrease in VCAM (p = 0.046), with 1496.89 ng/mL (IQR 1163.47–1597.77 ng/mL) at baseline and 1436.97 ng/mL (1315.89–1616.83 ng/mL) at four weeks after intervention (Figure 3).

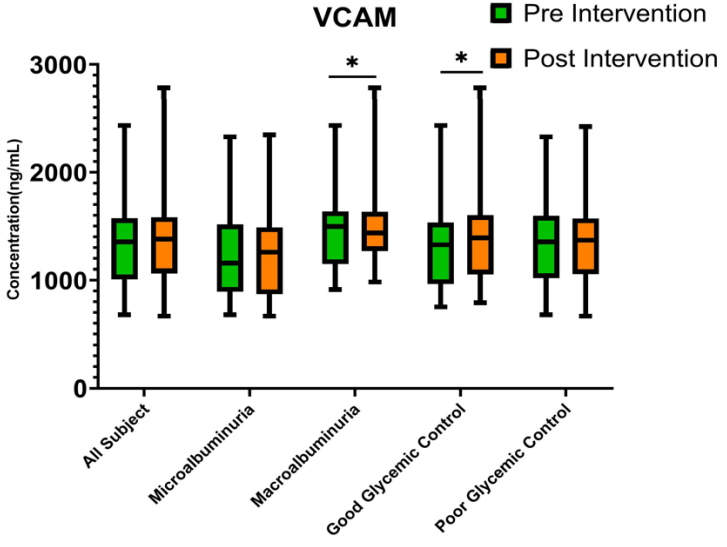

Figure 3. VCAM pre- and post intervention with sub-group analysis. * p < 0.05; hypothesis testing was conducted using the Wilcoxcon sign rank test. Microalbuminuria: UACR 30–300 mg/g at baseline; macroalbuminuria: UACR > 300 mg/g at baseline; good glycemic control: HbA1c ≤ 7.00; poor glycemic control: HbA1c > 7.00. VCAM: vascular cell adhesion molecule; total subjects n = 69, microalbuminuria n = 36, macroalbuminuria n = 36, good glycemic control n = 18, poor glycemic control n = 51.

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Table 2. Endothelial dysfunction biomarker (ICAM, VCAM, VEGF) levels.

Parameter	Pre Intervention	Post Intervention	*p*-Value
ICAM (ng/mL)	314.50 ± 93.12	315.12 ± 78.98	0.905
VCAM (ng/mL)	1354.25 (1015.90–1567.03)	1380.65 (1067.92–1572.03)	0.827
VEGF (pg/mL)	504.44 (294.54–790.72)	494.57 (319.39–775.10)	0.664

Hypothesis testing was conducted using the Wilcoxon sign test for VCAM and VEGF, and paired t-test for ICAM. VCAM and VEGF were presented as median (IQR). ICAM was presented as mean ± SD.

3.5. Correlation of Clinical Outcome Parameters with Endothelial Dysfunction Biomarkers

In order to validate the relationship between kidney function and endothelial biomarker, correlation analysis was conducted. Figure 4 shows the results of the correlation between kidney function and endothelial dysfunction biomarkers before and after autologous dendritic cell immunotherapy. Before immunotherapy, UACR showed a significant correlation with VCAM ($r = 0.346$; $p = 0.002$) and VEGF ($r = 0.252$; $p = 0.018$), while there was no significant correlation with ICAM ($r = 0.048$; $p = 0.349$). After immunotherapy, the correlation between UACR and VCAM remained significant, though it decreased ($r = 0.243$; $p = 0.022$). The correlation between UACR and VEGF slightly increased ($r = 0.376$; $p = 0.001$). However, UACR still did not show a significant correlation with ICAM after immunotherapy ($r = 0.057$; $p = 0.320$).

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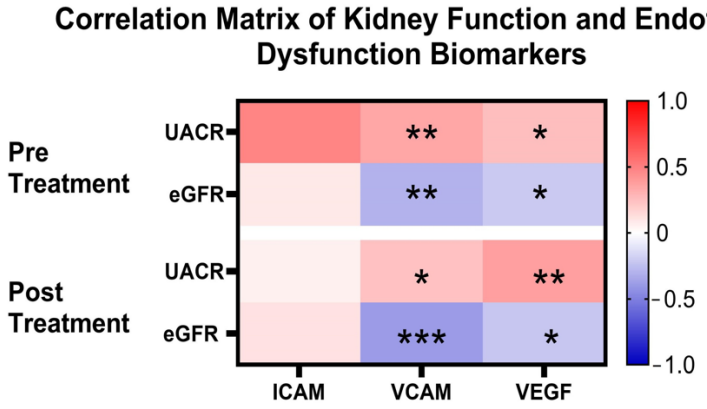

Figure 4. Correlation matrix of kidney function and endothelial dysfunction biomarkers. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; Correlational analysis was conducted using Spearman correlation. ICAM: intercellular adhesion molecule; VCAM: vascular cell adhesion molecule; VEGF: vasoendothelial growth factor.

Furthermore, before immunotherapy, eGFR showed a significant negative correlation with VCAM ($r = -0.304$; $p = 0.006$) and VEGF ($r = -0.213$; $p = 0.039$), while no significant correlation was found with ICAM ($r = 0.087$; $p = 0.240$). After immunotherapy, no meaningful changes in the correlations occurred. Although there was a slight increase in the negative correlation between eGFR and VCAM ($r = -0.395$; $p = 0.000$), this change remained significant. The negative correlation with VEGF also remained significant ($r = -0.225$; $p = 0.031$), indicating a consistent relationship between decreased eGFR and increased VEGF. The correlation between eGFR and ICAM remained non-significant post immunotherapy ($r = 0.115$; $p = 0.173$). These results indicate that endothelial dysfunction, as reflected by elevated levels of VCAM and VEGF, is linked to a decline in kidney function, as measured by eGFR and UACR, in DKD patients. The lack of significant changes in the strength of these correlations after immunotherapy suggests that the relationship remains largely unchanged.

4. Discussion

The majority of the subjects were in the elderly age group, which aligns with the high-risk population for chronic diseases such as hypertension and diabetes. This study population has a high risk for cardiovascular and kidney complications due to the high prevalence of hypertension, diabetes, obesity, and dyslipidemia. The combination of antihypertensive and antidiabetic treatments used reflects intensive efforts to control these risk factors. However, albuminuria and reduced eGFR indicates that subjects have already experienced significant kidney damage.

Subcutaneous injection was selected as the route of administration because the subcutaneous layer of the skin is rich in adipose tissue. Dendritic cells require migration to lymphatic organs to exert their full functionality. The initial dermal lymphatic vessels, which are open-ended, are located just above the adipose tissue layer in the skin [25]. Moreover, deposition into adipose tissue allows for a slower release of dendritic cells compared to the rapid flow characteristics of blood vessels like veins or arteries. This slow and sustained presence of DC is anticipated to increase the possibility of DC interaction with other immune cells.

This study showed a significant reduction in UACR after dendritic cell (DC) immunotherapy in DKD patients up to four weeks post intervention. Despite some weekly fluctuations, the overall median UACR remained lower compared to baseline. These results are comparable to standard therapies such as Telmisartan, Enalapril, GLP-1 RA, and SGLT2 inhibitors. These drugs can decrease UACR ranging from 17% to 65% after long-term use [26,27,28,29]. In this study, the analysis of the entire subject population revealed that DC immunotherapy reduced UACR by 25.41% ± 36.40% (mean ± standard deviation) at four weeks after intervention. Sub-group analysis revealed that the percentage of reduction between microalbuminuria and macroalbuminuria was not significantly different. This signifies that this treatment is effective in both subjects' characteristics.

HbA1c and baseline UACR were identified as significant predictors of UACR change. The target HbA1c level for diabetes mellitus (DM) patients is <7%, making this cutoff value applicable for classifying glucose control among subjects [30]. Previous studies have revealed that

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higher HbA1c levels correlate with increased albumin excretion rates [31]. Chronic hyperglycemia contributes to glomerular damage through mechanisms such as advanced glycation end-product (AGE) accumulation, oxidative stress, and inflammation, all of which exacerbate albuminuria [32]. Consequently, HbA1c serves not only as a marker of metabolic status but also as a modifiable factor in therapeutic strategies aimed at mitigating renal damage. Baseline UACR, on the other hand, provides a direct measure of kidney damage at the initiation of treatment. Elevated UACR levels indicate significant glomerular injury, increased permeability, and heightened inflammatory activity. Its predictive value for subsequent UACR changes suggests that the severity of initial kidney damage profoundly influences the response to therapy. However, the negative impact of baseline UACR on UACR improvement observed in this study implies that after a certain degree of kidney damage, therapeutic interventions may become less effective due to the irreversible nature of the injury. This study did not limit the use of drugs that might affect albuminuria. Concomitant used of RAAS inhibitor, SGLT-2 inhibitor, or GLP-1RA did not contribute significantly to UACR change.

The analysis of creatinine levels pre- and post immunotherapy did not show any significant differences. Similarly, eGFR values before and after immunotherapy did not differ significantly. Comparisons with previous studies revealed varying results regarding changes in eGFR and creatinine after therapy in patients with kidney disease. Some studies report that in chronic kidney disease patients, especially those with high levels of albuminuria, improvements in eGFR after intervention are often minimal or not statistically significant, despite observable trends [26,27]. These studies suggest that reductions in albuminuria and improvements in eGFR may take longer to achieve statistical significance. Typically, the effects of therapy on kidney function are more apparent after a longer treatment period and with more aggressive combination therapies.

The improvement in UACR observed in this study suggests an improvement in kidney function after DC immunotherapy. This improvement is hypothesized to be mediated by enhanced endothelial function, as indicated by improvements in ICAM, VCAM, and VEGF levels. Previous research has shown increased expression of ICAM and

VCAM in patients with kidney disorders, especially in diabetic kidney disease, supporting the hypothesis that these biomarkers play a role in the inflammatory process and endothelial damage [33,34]. Conversely, VEGF levels tend to be lower in DKD patients due to the loss of podocytes, the primary producers of VEGF in the kidney [35].

To confirm the relationship of endothelial biomarker with UACR, Spearman correlation analysis was conducted. Correlation analysis revealed that VCAM and VEGF have significant correlation with both UACR and eGFR. Our findings confirm previous studies which found that VCAM is a diagnostic biomarker of diabetic kidney disease progression that positively correlates with UACR and negatively correlates with eGFR [36]. VCAM is upregulated in response to inflammatory cytokines, which mediates immune cell infiltration and subsequent kidney damage [37]. On the other hand, VEGF is implicated in glomerular capillary hyperpermeability, contributing to diabetic albuminuria. In line with our findings, previous studies also found that a strong positive correlation exists between VEGF and UACR, indicating that higher VEGF levels are associated with increased albuminuria [37,38].

However, in this study, no significant changes were found in ICAM, VCAM, and VEGF biomarkers. While there were slight variations in median values, these changes were not substantial enough to be considered significant. This probably means that a decrease in UACR was not mediated by improvement of endothelial dysfunction. However, this should be interpreted with caution. There are other factors that should be considered. First, in comparison to previous studies analyzing changes in ICAM, VCAM, and VEGF, significant changes are often only seen after longer therapy durations or when combined with other interventions, such as improved glycemic control or additional pharmacological therapies directly affecting these biomarkers [39]. This suggests that the response to therapy may be cumulative and take longer to become evident in clinical trials. Second, previous studies have found that endothelial biomarkers like ICAM, VCAM, and VEGF tend to exhibit localized changes, which are not always reflected systemically [40]. In this study, biomarkers were measured from serum, representing systemic levels of ICAM, VCAM, and

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VEGF. However, it is possible that changes in these biomarkers were localized, making them undetectable in serum measurements.

Subgroup analysis revealed contrasting trends in VCAM changes: a decrease in VCAM levels among patients with macroalbuminuria and an increase in VCAM levels among those with good glycemic control. These findings suggest that the underlying mechanisms driving endothelial dysfunction and its resolution may differ depending on the baseline severity of kidney damage and glycemic control status. VCAM expression is upregulated in activated endothelial cells, facilitating the recruitment of inflammatory cells to sites of vascular damage [41]. In patients with macroalbuminuria, the observed decrease in VCAM may reflect a reduction in endothelial activation and inflammation following therapy, likely driven by the mitigation of severe vascular damage. This indicates that treatment may effectively reduce vascular inflammation in individuals with more advanced kidney damage. Conversely, the increase in VCAM levels in patients with good glycemic control could imply enhanced endothelial activity related to repair and regeneration rather than inflammation. VCAM enhances the migration and angiogenesis of bone marrow mesenchymal stem cells (BMSCs) which is important for repair and regeneration [42]. This group may exhibit an adaptive vascular response, where improved glycemic control supports endothelial recovery and function, leading to transient VCAM elevation.

This study has several limitations. First, this study evaluated changes in UACR, biomarkers and renal function within four weeks post immunotherapy. While significant reductions in UACR were observed, longer follow-up periods are required to assess sustained therapeutic effects, potential progression of kidney function, and delayed responses in biomarkers like ICAM, VCAM, and VEGF. Second, biomarkers like ICAM, VCAM, and VEGF were measured in serum, which reflects systemic levels. However, localized changes in these biomarkers within the kidney or specific vascular compartments may not be captured by systemic measurements, limiting the ability to fully assess endothelial dysfunction and repair mechanisms. Only a few endothelial biomarkers (ICAM, VCAM, and VEGF) were assessed. Other inflammatory and oxidative stress markers, which might provide a more comprehensive

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understanding of endothelial function and kidney health, were not included. Third, this study did not include a placebo or alternative therapy control group for direct comparison. While reductions in UACR were observed and compared with historical data on standard therapies, the absence of a control group limits the ability to attribute changes solely to dendritic cell immunotherapy. Multivariate analysis revealed that concomitant drug use did not significantly contribute to the UACR change. However, this does not exclude the possibility that these drugs might mask or even reduce the treatment effect of the intervention.

Future studies should address these limitations by incorporating longer follow-up periods, larger sample sizes, more comprehensive biomarker panels, and control groups. Such efforts will help validate the findings and provide deeper insights into the mechanisms and long-term efficacy of dendritic cell immunotherapy in diabetic kidney disease.

5. Conclusions

This study demonstrates that dendritic cell (DC) immunotherapy significantly reduces urinary albumin-to-creatinine ratio (UACR) in diabetic kidney disease (DKD) patients, suggesting its potential as an effective therapeutic approach. HbA1c and baseline UACR were identified as key predictors of UACR changes, highlighting the importance of glycemic control and baseline kidney damage in treatment outcomes. While no significant changes in endothelial biomarkers (ICAM, VCAM, and VEGF) were observed, the reduction in UACR indicates the involvement of other mechanisms, possibly localized endothelial repair or immune modulation. Subgroup analysis revealed contrasting VCAM trends, suggesting that endothelial responses vary based on glycemic control and the severity of kidney damage. These findings support the potential of DC immunotherapy for DKD, though further studies with longer follow-up and larger sample sizes are needed to validate and expand on these results.

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Clinical Trial: Effects of Autologous Dendritic Cell Administration on Renal Hemodynamics and Inflammatory Biomarkers in Diabetic Kidney Disease

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1. Introduction

Diabetic kidney disease (DKD) is a significant risk factor for the development of End-Stage Renal Disease (ESRD) [1]. Globally, an estimated 2.6 million new cases of DKD are diagnosed annually, and this number is projected to rise further [2]. The mortality rate among individuals with DKD is alarmingly high, reaching 31.1% worldwide [3].

In DKD, hyperglycemia induces a pro-inflammatory state, leading to elevated cytokine production and subsequent renal damage [4]. Inflammation affects renal hemodynamics, causing alterations in blood flow that initially result in glomerular hyperfiltration, a hallmark of early DKD progression [5]. Doppler ultrasound measurements, such as Peak Systolic Velocity (PSV) and End-Diastolic Velocity (EDV), provide non-invasive estimates of intraglomerular pressure, which are pivotal in understanding glomerular hyperfiltration [6]. Chronic inflammation further exacerbates kidney damage through fibrosis, which plays a central role in declining renal function. Studies suggest that the activation of MMP-9 facilitates the conversion of latent TGF-β into its active form, promoting fibrotic processes [7].

Recent discoveries suggest that autologous dendritic cell transfer holds promise as a therapeutic approach to alleviate inflammation [8,9,10]. These cells can be engineered ex vivo to enhance their immune-regulatory properties and subsequently reintroduced into a patient's body, promoting immune tolerance and mitigating inflammatory responses [11,12]. The therapeutic application of autologous dendritic cells has demonstrated potential in reducing inflammation and fibrosis across various conditions, positioning them as a compelling candidate for managing DKD [13,14]. This study aims to evaluate the effects of autologous dendritic cell administration on renal hemodynamics and fibrosis biomarkers, exploring their potential to address the challenges of DKD treatment.

2. Materials and Methods

2.1. Study Design

This study utilized a quasi-experimental design using a one-group pretest–post-test approach. This research was conducted at the Army Central Hospital with participants selected through a nonprobability sampling technique. Ethical approval for this study was granted by the Ethics Committee of the Army Hospital (Ethical Clearance No. 109/VIII/KEPK/2024, dated 23 August 2024). All participants provided written informed consent prior to their inclusion in this study. The clinical trial is registered with ClinicalTrials.gov under the trial registration number NCT06866158. The trial was first submitted on 22 February 2025.

2.2. Research Subject

A total of 10,930 subjects were initially identified from polyclinics within the hospital, with 1280 subjects from the endocrine clinic and 312 from the renal clinic. Among them, 36 subjects agreed to participate in this study. After applying of the exclusion criteria, 29 subjects completed this study and underwent ultrasound evaluations.

The inclusion criteria were required to meet the diagnostic criteria for Diabetes Mellitus according to the PERKENI 2021 guidelines, be over 18 years of age, provide written informed consent, and have an eGFR of ≥30 mL/min/1.73 m2 and a Urinary Albumin-to-Creatinine Ratio (UACR) of ≥ 30 mg/g. Additionally, the subjects needed to demonstrate an understanding of and willingness to comply with study procedures.

The exclusion criteria included receiving immunosuppressive therapy within the past four weeks, a history of other kidney diseases, alternative Diabetes Mellitus diagnoses, a positive pregnancy test, prior thromboembolism or genetic predisposition to thromboembolism, and use of anti-thromboembolic therapies other than low-dose aspirin. Subjects were also excluded if they had physical or mental disabilities preventing daily activities, medical conditions that could interfere with this study (e.g., acute, subacute, intermittent, or chronic conditions posing risk), excessive obesity (BMI > 40), or uncontrolled hypertension (systole > 180 mmHg, diastole > 100 mmHg) or were unwilling to provide written

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informed consent. These criteria ensured a carefully selected cohort for robust and reliable study outcomes.

2.3. Research Procedure

The research procedure began with the preparation of the subjects, which included collecting blood samples for baseline measurements of MMP-9 and TGF-β using sandwich ELISA kits (Reed Biotech Ltd., Wuhan, China) and for the generation of autologous dendritic cells. Additional procedures involved urine collection and Doppler ultrasound assessment of renal blood flow parameters: specifically, Peak Systolic Velocity (PSV) and End-Diastolic Velocity (EDV). The Doppler ultrasound examinations, performed by two specialist radiologists using a Siemens Acuson Sequoia ultrasound machine, focused on the interlobar arteries of the right and left kidneys, with the results averaged for both kidneys.

One week after the initial assessments, the autologous dendritic cells were injected subcutaneously into each subject's arm. Four weeks following the injection, the cytokine levels (MMP-9 and TGF-β) were reassessed, along with the renal blood flow velocities (PSV and EDV), using the same Doppler ultrasound methodology. This comprehensive approach ensured the accurate and consistent evaluation of both biochemical and hemodynamic changes throughout this study.

2.4. Autologous Dendritic Cell Generation

At baseline, 40 cc of blood was drawn from each subject. The blood was processed and incubated in a medium containing GM-CSF (Granulocyte Macrophage Colony Stimulating Factor) and IL-4 for five days to generate the dendritic cells. Subsequently, the antigen was added and incubated for two additional days to induce the maturation of the dendritic cells. Finally, the prepared autologous dendritic cells were administered by subcutaneous injection into each subject's arm.

2.5. Safety Evaluation

The injections were administered by a qualified physician, with the subjects monitored for 30 min after injection to observe any potential allergic reactions. Any adverse events meeting the criteria outlined in

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CTCAE v6.0 were documented for up to seven days following the injection. This study adhered strictly to both local and international regulations, aligning with the principles of the Declaration of Helsinki.

2.6. Statistics

A data normality test was conducted on each variable. The Shapiro–Wilk normality test was used for samples below 50, while the Kolmogorov–Smirnov test was used for samples above 50. The PSV and EDV variables were analyzed using a paired t-test for normally distributed data, while the non-normally distributed data were analyzed using the Wilcoxon signed-rank test. The MMP-9 and TGF-β variables used linear regression analysis tests to see the effects before and after the administration of the autologous dendritic cells.

2.7. GenAi Disclosure

This manuscript has been reviewed and enhanced with the assistance of a generative AI tool (Grammarly) to improve the language clarity, grammar, and overall readability. The tool was used solely for editorial purposes and does not alter the scientific content or methodology of this study. All decisions regarding the study design, data interpretation, and conclusions remain the responsibility of the authors.

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3. Results

3.1. Subject Characteristics

Table 1 presents the characteristics of the research subjects, comprising 29 participants. The majority of subjects were aged over 60 years. In terms of gender distribution, there were slightly more females (16) than males (13). Hypertension was the most prevalent comorbidity, affecting 96.6% of the participants. Regarding antidiabetic medication use, insulin was the most commonly utilized drug, reported by 69% of subjects. Similarly, angiotensin receptor blockers (ARBs) were the most frequently used antihypertensive medication, with 72.4% of participants reporting their use.

Table 1. Characteristics of research subjects.

		Count	Table N %
Gender	Women	16	55.2%
	Men	13	44.8%
Age	<60	9	31.0%
	≥60	20	69.0%
BMI	Underweight	2	6.9%
	Normal weight	6	20.7%
	Overweight	0	0.0%
	Obesity I	13	44.8%
	Obesity II	8	27.6%
Hypertension	No	1	3.4%
	Yes	28	96.6%
Stroke	No	24	82.8%
	Infarction	5	17.2%
	Hemorrhagic	0	0.0%
Heart disease	No	19	65.5%
	Yes	10	34.5%
Retinopathy	No	25	86.2%
	Yes	4	13.8%
Neuropathy	No	13	44.8%
	Yes	16	55.2%
Biguanid	No	20	69.0%
	Yes	9	31.0%
Thiazolidinedione	No	29	100.0%
	Yes	0	0.0%
Glinid	No	29	100.0%
	Yes	0	0.0%
α glucosidase Inhibitor	No	26	89.7%

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		Count	Table N %
	Yes	3	10.3%
Insulin	No	9	31.0%
	Yes	20	69.0%
Gliptin	No	23	79.3%
	Yes	6	20.7%
SGLT2	No	26	89.7%
	Yes	3	10.3%
Sulphonylurea	No	17	58.6%
	Yes	12	41.4%
Central alpha agonist	No	29	100.0%
	Yes	0	0.0%
Diuretics	No	27	93.1%
	HCT	2	6.9%
	Spironolacton	0	0.0%
Alpha-blockers	No	28	96.6%
	Yes	1	3.4%
CCB	No	9	31.0%
	Dihydropyridine	15	51.7%
	Non- Dihydropyridine	5	17.2%
	DHP AND NON DHP	0	0.0%
β Blockers	No	21	72.4%
	Yes	8	27.6%
ARB	No	8	27.6%
	Yes	21	72.4%

Abbreviations: HCT = Hydrochlorothiazide, CCB = Calcium Channel Blocker, ARB = Angiotensin II Receptor Blocker.

3.2. PSV and EDV Results

Table 2 shows the changes in the PSV and EDV. The autologous dendritic cell administration showed significant changes in the PSV and EDV parameters. Before the dendritic cell administration, the median PSV value was 47.1 ± 23.87 cm/s. After the dendritic cell administration, the median PSV value decreased to 27.85 ± 20.53 cm/s. This decrease was statistically significant, with a p-value of 0.044. The median EDV value before the administration was 13 ± 5.32 cm/s. After the dendritic cell administration, the median EDV value decreased to 15.7 ± 12.55 cm/s. This decrease was statistically significant, with a p-value of 0.039.

Table 2. Results of PSV and EDV analysis before and after autologous dendritic cell administration.

	Before (cm/s)	After (cm/s)	*p*-Value
PSV (Median ± IQR)	47.1 ± 23.87	27.85 ± 20.53	0.044
EDV (Median ± IQR)	13 ± 5.32	15.7 ± 12.55	0.039

Abbreviations: PSV = Peak Systolic Velocity, EDV = End-Diastolic Velocity, IQR = Interquartile Range.

Table 3 shows the PSV values based on gender, age, and UACR. In the male group, the median PSV value before the dendritic cell administration was 47.77 ± 14.96 cm/s, and after the dendritic cell administration, the median value decreased to 27.05 ± 42.38 cm/s, although this was not statistically significant (p = 0.422). In the female group, there was a significant decrease from 51.65 ± 24.8 cm/s to 31.72 ± 18.31 cm/s with a p-value = 0.03.

Table 3. PSV by sex, age, and UACR before and after autologous dendritic cell administration.

		PSV Before (cm/s)	PSV After (cm/s)	*p*-Value
Gender *	Men	47.1 ±23.3	27.05 ±42.38	0.422
	Women	51.65 ±24.8	31.72 ±18.31	0.03
Age †	<60	52.56 ±18.41	42.32 ±24.80	0.225
	≥60	47.02 ±24.97	29 ±20.43	0.121
UACR *	Microalbuminuria	54.6 ±23.46	27.65 ±16.74	0.011
	Macroalbuminuria	47.05 ±32.3	35.7 ±32.28	0.834

Abbreviations: PSV = Peak Systolic Velocity, IQR = Interquartile Range, SD = Standard Deviation, UACR = Urinary Albumin-to-Creatinine Ratio. * Median ± IQR. † Mean ± SD.

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In the age group of below 60 years, the mean value of the PSV before the dendritic cell administration was 52.56 ± 18.41 cm/s, and after the dendritic cell administration, the mean value decreased to 42.32 ± 24.80 cm/s, although this was not statistically significant (p = 0.225). In the age group of above 59 years, there was a decrease in the median value from 47.02 ± 24.97 cm/s to 29 ± 20.43 cm/s with a p-value = 0.121.

Changes in the PSV were also found in the UACR group before and after the administration of the autologous dendritic cells. In the microalbuminuria group, the PSV before the administration of the autologous dendritic cells had a median value of 54.6 ± 23.46 cm/s. The PSV after the autologous dendritic cell administration had a median value of 27.65 ± 16.74 cm/s. Hypothesis testing with a p-value of 0.011 showed that this is a significant difference. In the macroalbuminuria group, the median value of the PSV before the administration of the autologous dendritic cells was 47.05 ± 32.3 cm/s. The PSV after the administration of the autologous dendritic cells had a median value of 35.7 ± 32.28 cm/s. Hypothesis testing showed that this change was not significant, with a p-value of 0.834.

Table 4 shows the EDV analysis based on gender, age, and UACR group. In the EDV analysis based on gender, the results show that in the male group, the median EDV value before the dendritic cell administration was 12.55 ± 6.97 cm/s. After the dendritic cell administration, there was an increase to 15.7 ± 21.9 cm/s. This increase is not statistically significant, with a p-value of 0.249. In the female group, the median EDV value before the dendritic cell administration was 13.27 ± 6.8 cm/s. After the dendritic cell administration, there was a significant increase to 15.04 ± 11.08 cm/s, with a p-value of 0.044.

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Table 4. EDV by sex, age, and UACR before and after autologous dendritic cell administration.

		EDV Before (cm/s)	EDV After (cm/s)	*p*-Value
Gender *	Men	12.55 ±6.97	15.7 ±21.9	0.249
	Women	13.27 ±6.8	15.04 ±11.08	0.044
Age †	<60	15.53 ±6.10	23.03 ±14.93	0.137
	≥60	4.11 ±6.08	12.64 ±11.08	0.126
UACR *	Microalbuminuria	13.8 ±5.36	14.19 ±11.18	0.234
	Macroalbuminuria	11.15 ±6.28	16.4 ±17.75	0.087

Abbreviations: EDV = End-Diastolic Velocity, IQR = Interquartile Range, SD = Standard Deviation, UACR = Urinary Albumin-to-Creatinine Ratio. * Median ± IQR. † Mean ± SD.

In the age group of below 60 years, the mean value of the EDV before the administration of the autologous dendritic cells was 15.53 ± 6.10 cm/s. After the administration of the autologous dendritic cells, there was an increase in the mean value of the EDV by 23.03 ± 14.93 cm/s. This increase was not statistically significant, with a p-value of 0.137. In the age group of above 60 years, the median value before the administration of the autologous dendritic cells was 4.11 ± 6.08 cm/s. After the administration of the autologous dendritic cells, there was an increase in the median value of 12.64 ± 11.08 cm/s. This increase was not statistically significant, with a p-value of 0.126.

The EDV in the microalbuminuria group had a median value of 13.8 ± 5.36 cm/s before the autologous dendritic cell administration; after the autologous dendritic cell administration, the median value increased to 14.19 ± 11.18 cm/s. This increase was not statistically significant, with a p-value of 0.234. In the macroalbuminuria group, the median value before the administration of the autologous dendritic cells was 11.15 ± 6.28 cm/s.

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After the administration of the dendritic cells increased to 16.4 ± 17.75 cm/s, this increase was not statistically significant, with a p-value of 0.234.

3.3. TGF-β and MMP-9 Results

Table 5 shows the linear regression testing of the TGF-β and MMP-9 before and after the administration of the autologous dendritic cells. The linear regression test before the action showed that every increase of one unit of MMP-9 would increase the TGF-β by 13.112, and this result was close to significant, with a p-value = 0.058. The linear regression test after the treatment showed that every one unit increase in MMP-9 would increase the TGF-β by 7.622, with a near significant p-value (p-value = 0.066). However, when comparing the value of the MMP-9 to the TGF-β before and after the autologous dendritic cell administration, there was a decrease in the value of the MMP-9 to that of the TGF-β.

Table 5. Linear regression testing of TGF-β and MMP-9 before and after autologous dendritic cell administration.

Variables	Coefficient (β)	*p*-Value
MMP-9 Before Dependent Variable TGF-β Before	13.112	0.058
MMP-9 After Dependent Variable TGF-β After	7.622	0.066

Abbreviations: MMP-9 = Matrix Metalloproteinase-9, TGF-β = Transforming Growth Factor Beta.

Table 6 shows the analysis of the relationship between the study variables. The variables before and after the administration of the autologous dendritic cells were combined, and then the correlation test between the variables was performed.

Table 6. Analysis of the relationship between variables.

Variables	MMP-9 (r, *p*)	PSV (r, *p*)	EDV (r, *p*)
TGF-β	0.413, 0.001 **	−0.101, 0.452	−0.071, 0.598
MMP-9	-	−0.015, 0.909	−0.048, 0.721
PSV	−0.015, 0.909	-	0.675, 0.000 **
EDV	−0.048, 0.721	0.675, 0.000 **	-

Abbreviations: MMP-9 = Matrix Metalloproteinase-9, TGF-β = Transforming Growth Factor Beta, PSV = Peak Systolic Velocity, EDV = End-Diastolic Velocity. r = correlation coefficient. Note: ** $p < 0.01$.

The relationship test between the research variables using Spearman showed that there was a significant relationship between the TGF-β and MMP-9, with a p-value of 0.001. There was also a significant relationship between the PSV and EDV, with a p-value of 0.000.

4. Discussion

A significant portion of the study population (93.1%) had a history of hypertension, a known major risk factor for DKD. This aligns with the existing literature, where hypertension is recognized as one of the most significant contributors to the progression of kidney disease [15]. The pathophysiology of hypertension in DKD involves the activation of the vasoactive hormone pathway, leading to glomerular hyperfiltration, increased glomerular pressure, and subsequent inflammation and fibrosis [16].

In this cohort, angiotensin receptor blockers (ARBs) were the most commonly prescribed antihypertensive medication. Research supports the use of ARBs and ACE inhibitors in reducing Urinary Albumin-to-Creatinine Ratios (UACRs) and improving renal function in DKD patients [17]. However, as noted, combining ARBs with mineralocorticoid receptor antagonists can increase the risk of hyperkalemia [18].

This study showed that there were no significant differences between the UACR groups for PSV, EDV, TGF-β, and MMP-9 before the

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administration of the autologous dendritic cells. In addition, there were no significant differences based on the history of the disease or the use of diabetic drugs or a history of antihypertensive drug use.

Significant changes in the PSV and EDV were observed following the dendritic cell therapy, indicating improvement in renal blood flow and perfusion. PSV and EDV are important hemodynamic indicators of renal artery resistance, with a higher PSV often correlated with increased renal resistance and a pro-inflammatory state. Several studies have shown that increased PSV is associated with elevated levels of C-reactive protein (CRP), a marker of inflammation, especially in hypertensive and diabetic patients [19]. Similarly, a higher PSV typically correlates with a lower GFR, as increased vascular resistance impairs kidney filtration. This is due to the fact that higher PSVs reflect reduced renal perfusion and greater resistance in the blood vessels. Conversely, a higher EDV usually correlates with a higher GFR [20].

The reduction in the PSV was also related to the UACR and eGFR, which are established markers of kidney function. An increase in the UACR is a sign of early kidney damage and is typically associated with a decline in the eGFR, which measures the glomerular filtration rate and reflects renal function [21]. Some studies explain that microalbuminuria is associated with high PSV [20].

The increase in the EDV after the dendritic cell therapy is also noteworthy. EDV is a measure of renal perfusion and is positively correlated with the glomerular filtration rate (GFR), a key marker of kidney function. Research indicates that a decrease in EDV will lead to increased vascular resistance, impaired perfusion, and reduced GFR [22]. In kidney disease, this decrease in the EDV will increase resistance in blood vessels and cause impaired perfusion and a decreased GFR [23]. Decreased EDV will also lead to increased albuminuria through a decreased GFR [24]. Some studies also explain the existence of an immune response, namely inflammation, in patients with microalbuminuria and macroalbuminuria [25].

Regarding gender-specific differences, our results indicate that significant changes in PSV and EDV were observed in the female group, while the

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men did not show significant changes. This finding may be explained by differences in immune responses between the genders, with women generally exhibiting stronger immune responses, possibly due to the influence of estrogen, which can enhance antibody production [26]. This finding indicates a potential gender-specific response and the need for further investigation in larger cohorts.

The differences in renal physiology and immune response based on age are the basis for the grouping in this study. This is in line with research conducted by Costagliola et al., which showed differences in immune responses based on age [27]. Research conducted by Weinstein et al. shows that there are changes in renal blood flow with age [28]. However, in this study, in both the age groups of under 60 and over 60 years, there were no significant changes in PSV and EDV.

We also explored the pathophysiological mechanisms of microalbuminuria and macroalbuminuria. Microalbuminuria is an early marker of kidney damage, whereas macroalbuminuria often indicates significant structural changes in the glomerulus [29,30]. In the microalbuminuria group, the PSV was significantly reduced after the dendritic cell therapy, whereas the EDV showed an increase, though not statistically significant. These findings suggest that early-stage DKD may respond more effectively to DC therapy, whereas advanced stages like macroalbuminuria, which involve substantial glomerular damage, may require more intensive treatment or longer follow-up periods to observe improvements in renal hemodynamics.

Linear regression analysis showed a reduction in the influence of MMP-9 on TGF-β after dendritic cell therapy, although this result approached statistical significance ($p = 0.066$). The difference between the Spearman test (showing an association between TGF-β and MMP-9) and the linear regression findings may be attributed to the small sample size, which limited the statistical power. Previous research has highlighted the interaction between TGF-β and MMP-9, showing that TGF-β activates MMP-9, which in turn promotes fibrogenesis [31]. Research conducted by Gu et al. showed that inhibition of TGF-β can reduce MMP-9 [32]. Muscella et al. stated that TGFβ activates cell migration through MMP 2

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and MMP-9 [33]. In this study, there was a decrease in the influence of MMP-9 on TGF-β after the administration of the autologous dendritic cells. The decrease in the TGF-β was expected to reduce fibrosis in the kidney. TGF-β activates Smad2 and Smad 3 and then interacts with the transcription factors involved in fibrogenesis [34,35].

5. Conclusions

Autologous dendritic cell administration can positively impact renal hemodynamics in patients with DKD. Significant changes were observed in PSV and EDV, with improvements indicating enhanced renal blood flow. Notably, autologous dendritic cells were effective in reducing inflammation and fibrosis markers, particularly in the microalbuminuria group, suggesting a potential therapeutic benefit. Although no significant changes were seen in the macroalbuminuria group, longer treatment and follow-up may be required for patients with more advanced kidney damage. While the evidence for fibrosis reduction is promising, further studies with larger cohorts and more direct measures of fibrosis are required to confirm the therapeutic potential of DC therapy for DKD.

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The Effect of Autologous Dendritic Cell Therapy on Renal Perfusion in Diabetic Kidney Disease: Analysis of Doppler Ultrasound and Angiogenesis Biomarkers

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1. Introduction

Type 2 diabetes is a complex chronic disease caused by insulin resistance or insufficient insulin production, closely associated with lifestyle and environmental factors. It has become one of the fastest-growing global health concerns [1]. The prevalence of DM type 2 in Indonesia has reached 10.8% in 2021, positioning the country within the top 10 for the highest diabetes prevalence worldwide. It is estimated that approximately 463 million adults aged 20–79 years suffer from DM type 2, while the number is projected to rise to 578 million by 2030 [2]. Previous studies indicate that around 40% of type 2 diabetes patients develop complications, such as DKD, with over half suffering from moderate-to-severe (stages 3 and 4) DKD [3].

DKD is typically caused by microvascular damage related to diabetes, and it is one of the leading causes of kidney failure globally. DKD is typically defined by persistent albuminuria (urinary albumin excretion of more than 30 mg/24 h or a urine albumin-to-creatinine ratio exceeding 30 mg/g), a sustained reduction in the estimated glomerular filtration rate (eGFR) less than 60 mL/min per 1.73 m2, or a combination of both for at least three months [3]. The pathophysiology of DKD is highly complex, involving metabolic, hemodynamic, inflammatory, and fibrotic pathways that collectively drive oxidative stress and compromise renal perfusion. The hemodynamic component, which is mediated by the renin–angiotensin–aldosterone system (RAAS), angiotensin II, endothelin-1, and the buildup of advanced glycation end-products (AGEs), is a crucial part of this process. These factors collectively act to worsen renal hypoperfusion, further complicating the disease's progression [4].

Radiological modalities, such as Doppler ultrasound, can be utilized to assess renal perfusion. The essential parameters for evaluating intrarenal hemodynamics are the RI and PI [5]. Measuring the RI and PI of the renal interlobar arteries is essential in assessing intrarenal perfusion in DKD, as they can describe intrarenal vasoconstriction that may predict complications in the future [6].

Furthermore, previous studies have suggested that angiogenesis markers, such as vascular endothelial growth factor (VEGF) and endothelin, are

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emerging indicators for predicting the progression of diabetic nephropathy due to their close association with renal perfusion [7]. Endothelin is essential in vasoconstriction, inflammation, and kidney fibrosis, which can negatively impact glomerular filtration and increase proteinuria levels. Meanwhile, VEGF is essential in maintaining renal perfusion in both standard and pathological conditions. It is crucial in angiogenesis to sustain the microvascular structure of the kidney [8,9,10,11].

Our previous study revealed that administering autologous dendritic cells can significantly reduce UACR in DKD patients. Decreases in TNF-α, a potent driver of inflammation, were also found after the administration of autologous dendritic cells, which strongly suggests the cells can induce inflammation controls that eventually lead to an improvement in UACR [12]. However, the analysis of endothelial biomarkers (ICAM, VCAM, and VEGF) revealed differential responses in specific subgroups, mainly based on the classification of albuminuria and glycemic status [13]. Furthermore, water molecule diffusion was measured using Magnetic Resonance Imaging (MRI) to assess whether the treatment induced structural changes in the kidney. However, no significant changes were detected [14]. This finding suggests that the reduction in albuminuria following autologous dendritic cell administration was not attributable to improvements in kidney structure as indicated by water molecule diffusion. Their impact on renal perfusion and angiogenesis markers needs to be examined to further elucidate the mechanism of action underlying the therapeutic effect of autologous dendritic cells. Hence, this study aims to analyze the differences in renal perfusion in DKD patients by using ultrasound to assess changes in RI and PI, as well as differences in angiogenesis markers, including VEGF and endothelin, before and after autologous dendritic cell administration.

2. Materials and Methods

2.1. Study Design

This study constitutes experimental research utilizing a quasi-experimental design, specifically a one-group pre-test post-test design, in which no randomization or blinding was applied. The procedures were conducted according to applicable regulations. The protocols were

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approved by the Ethics Committee of the Indonesia Central Army Hospital Gatot Soebroto (RSPAD) with ethical clearance letter number 101/VIII/KEPK/2024. This study is registered at ClinicalTrials.gov with the identifier NCT06866158. All subjects provided written informed consent before participation.

2.2. Study Subjects

The study sample comprised all DKD patients at RSPAD who qualified for the inclusion criteria. These criteria included a diagnosis of type 2 diabetes mellitus (DM) based on Indonesian guidelines, an eGFR of ≥30 mL/min/1.73 m2, and a urinary albumin-to-creatinine ratio (UACR) of ≥30 mg/g for the proteinuria group. The exclusion criteria were patients undergoing immunosuppressive therapy, those with other kidney diseases or conditions causing proteinuria, patients with different types of DM, positive pregnancy tests, individuals with immunodeficiency disorders, those with invasive cancer receiving non-hormonal treatment, patients with a history of thromboembolism, or those with physical or mental disabilities limiting daily activities, as well as any medical condition that would hinder participation.

2.3. Study Protocol

Each subject participated in a five-week clinical trial, starting with the screening phase, laboratory examinations, Doppler ultrasound assessments, blood collection for autologous dendritic cell preparation, and autologous dendritic cell injections. Laboratory assessments and Doppler ultrasound evaluations were conducted four weeks after the autologous dendritic cell injection.

2.4. Laboratory Examination

Angiogenesis biomarkers that affect renal perfusion, namely VEGF and endothelin, were measured using a sandwich ELISA (Reed Biotech Ltd., Wuhan, China) from serum blood samples collected before and four weeks after the autologous dendritic cell injection.

2.5. Doppler Ultrasound Examination

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Doppler ultrasound assessments were performed supine, with Siemens Acuson NX3® ultrasound utilizing a curvilinear probe in spectral Doppler mode, with an insonation angle of less than 60° between the transducer and blood flow. These assessments measured the RI and PI of the right and left interlobar renal arteries. Images obtained from the examinations are illustrated in Figure 1. Two radiologists conducted the examinations before the autologous dendritic cell injection and four weeks afterward.

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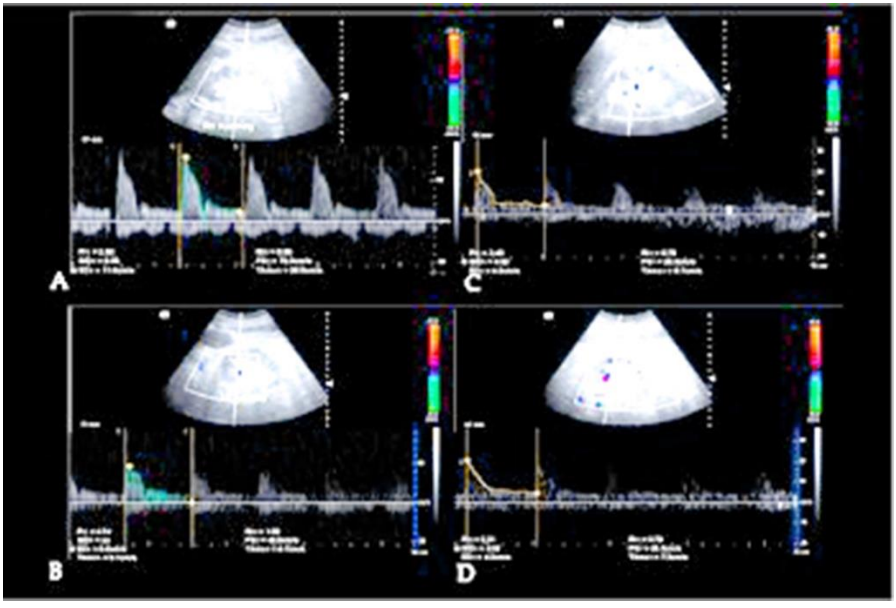

Figure 1. This picture illustrates Doppler ultrasound findings demonstrating changes in the resistive index (RI) and pulsatility index (PI) in diabetic kidney disease (DKD) patients following autologous dendritic cell administration. The Doppler ultrasound was performed on the interlobar arteries of both kidneys to assess RI and PI. Pre-treatment (A,B), the right kidney exhibited an RI of 0.86 and a PI of 2.50, while the left had an RI of 1.00 and a PI of 4.74. Post-treatment (C,D), RI and PI decreased bilaterally, with the right kidney showing an RI of 0.79 and a PI of 2.40 and the left kidney demonstrating an RI of 0.79 and a PI of 2.21.

2.6. Dendritic Cells (DC) Preparation

A total of 40 cc of peripheral blood was collected from each subject, from which monocytes were isolated using LymphoprepTM (StemcellTM Technologies Inc., Vancouver, BC, Canada). Granulocyte Macrophage Colony Stimulating Factor and Interleukin-4 (Aivita Biomedical, Irvine, CA, USA) were added to the culture medium for five days at 37 °C with 5% CO2. Then, antigens were added for two days to initiate maturation (Aivita Biomedical, Ievine, CA, USA). We did not separate monocytes and lymphocytes during the culture process to allow lymphocyte activation. As a result, the final cell product consisted of a mixed

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population of dendritic cells and lymphocytes. The flow cytometry (FACS) results demonstrating the cell product specification are provided in Supplementary Figure S1.

2.7. Statistical Analysis

Data analysis was conducted using either an independent t-test or a Wilcoxon test, which was selected based on the outcome of the normality test. All statistical analyses were performed using the Statistical Packages for Social Science (SPSS®) software version 25.

3. Results

3.1. Subject Characteristic

The study involved 35 participants, of which 22 were female (62.9%) and 13 were male (37.1%). The majority of the participants were over the age of 60, with 23 individuals in this category, while 12 were under the age of 60. The median body weight was 65 kg, and the median height was 158 cm. The participants had normal albuminuria, with 21 (60.0%) presenting with microalbuminuria and 14 (40.0%) with macroalbuminuria. The severity of CKD was also assessed, with 16 participants (45.7%) in CKD stages 1 and 2, while 19 participants (54.3%) were in CKD stage 3 (Table 1).

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Table 1. Subject characteristics. Gender, age group, body mass index classification, UACR classification, and CKD grade are given as n (%), while age, weight, and height are given as the median (min–max).

Characteristics	Category	Description (*n* = 35)
Gender	Female	22 (62.9%)
	Male	13 (37.1%)
Age group	<60	12 (34.2%)
	>60	23 (65.7%)
Age		62 (44–83) years
Weight		65 (41–101) kg
Height		158 (145–181) cm
Body mass index classification	Underweight	2 (5.7%)
	Normal weight	9 (25.7)
	Overweight	0 (0%)
	Obesity I	14 (40%)
	Obesity II	10 (28.6%)
UACR classification	Normal albuminuria	0 (0%)
	Microalbuminuria	21 (60%)
	Macroalbuminuria	14 (40%)
CKD grade	Stages 1 and 2	16 (45.7%)
	Stage 3	19 (54.3%)

3.2. Resistive Index (RI) and Pulsatility Index (PI) Changes

This study assessed renal perfusion using Doppler ultrasound, measuring the RI and PI at two points, before and four weeks after autologous dendritic cell administration. A comprehensive Doppler ultrasound evaluation was performed for each subject to ensure measurement consistency.

The overall mean ± standard deviation (SD) of RI before treatment was 0.74 ± 0.07, which increased to 0.75 ± 0.06 four weeks post-treatment, though this change was not statistically significant ($p = 0.17$). Subgroup analysis based on urinary albumin-to-creatinine ratio (UACR) showed that

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in the microalbuminuria group, the mean ± SD RI was 0.74 ± 0.07 before treatment and 0.75 ± 0.07 after treatment (p = 0.215). In the macroalbuminuria group, the pre-treatment RI was 0.74 ± 0.07, increasing to 0.76 ± 0.04 post-treatment (p = 0.465).

Further stratification by chronic kidney disease (CKD) stage revealed that in patients with CKD stages 1 and 2, the mean ± SD RI was 0.73 ± 0.05 pre-treatment and 0.74 ± 0.05 post-treatment (p = 0.295). Among those with CKD stage 3, the RI increased from 0.75 ± 0.08 pre-treatment to 0.76 ± 0.06 post-treatment (p = 0.327). These results indicate that while a slight increase in RI was observed across all groups, the changes were not statistically significant (Table 2).

Table 2. Resistive index (RI). Data are presented as median ± interquartile range. Wilcoxon signed rank calculated p-value.

Subject Group		Pre-Autologous Dendritic Cells	Post-Autologous Dendritic Cells	*p*-Value
Overall		0.74 ± 0.07	0.75 ± 0.06	0.17
UACR	Microalbuminuria	0.74 ± 0.07	0.75 ± 0.07	0.215
	Macroalbuminuria	0.74 ± 0.07	0.76 ± 0.04	0.465
Stage CKD	CKD stages 1 and 2	0.73 ± 0.05	0.74 ± 0.05	0.295
	CKD stage 3	0.75 ± 0.08	0.76 ± 0.06	0.327

Doppler ultrasound assessed the PI before and four weeks after autologous dendritic cell administration. Overall, the pre-treatment PI had a median of 1.61 ± 0.63, significantly decreasing to 1.21 ± 0.26 post-treatment (p < 0.001).

Subgroup analysis based on urinary albumin-to-creatinine ratio (UACR) categories showed that in the microalbuminuria group, the median PI decreased from 1.57 ± 0.49 pre-treatment to 1.22 ± 0.50 post-treatment, with a statistically significant p-value of <0.001. In the macroalbuminuria group, the mean ± standard deviation (SD) PI was 1.83 ± 0.67 pre-treatment, significantly decreasing to 1.23 ± 0.14 post-treatment (p = 0.004).

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Stratification based on CKD stages revealed that in CKD stages 1 and 2, the mean ± SD PI decreased from 1.58 ± 0.31 pre-treatment to 1.15 ± 0.15 post-treatment ($p < 0.001$). Among patients with CKD stage 3, the median PI was 1.61 ± 0.83 pre-treatment, significantly decreasing to 1.29 ± 0.423 post-treatment ($p = 0.002$). These findings indicate a significant reduction in PI across all subgroups following autologous dendritic cell therapy (Figure 2).

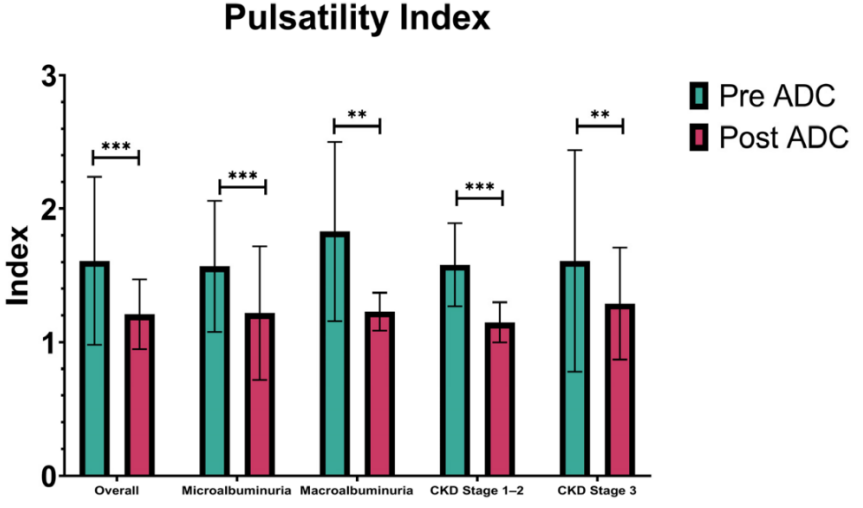

Figure 2. Pulsatility index. Data from overall, microalbuminuria, and CKD stage 3 are presented as the median ± IQR, and p-values were calculated by Wilcoxon signed rank. Meanwhile, data from macroalbuminuria and CKD Stages 1–2 are presented as the mean ± standard deviation, and the p-value was calculated by paired t-test. ** $p < 0.01$, *** $p < 0.001$. ADC: autologous dendritic cell, CKD: chronic kidney disease.

3.3. Angiogenesis Biomarker Changes

Angiogenesis biomarkers, including vascular endothelial growth factor (VEGF) and endothelin, were measured in this study. Each subject underwent comprehensive laboratory testing for VEGF and endothelin at

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two points before and four weeks after autologous dendritic cell administration.

VEGF levels were analyzed before and after treatment. In the overall analysis, the median VEGF level was 522.10 ± 608.6 pg/mL pre-treatment, slightly decreasing to 473.70 ± 550 pg/mL post-treatment. However, this change was not statistically significant (p = 0.589).

Subgroup analysis based on the urinary albumin-to-creatinine ratio (UACR) showed that in the microalbuminuria group, the pre-treatment VEGF median was 305.05 pg/mL, increasing slightly to 318.10 pg/mL post-treatment (p = 0.913). In the macroalbuminuria group, the mean ± standard deviation (SD) VEGF level was 611.72 ± 319.10 pg/mL pre-treatment, decreasing marginally to 604.35 ± 295.19 pg/mL post-treatment, with no significant difference (p = 0.863).

When stratified by CKD stage, patients with CKD stages 1 and 2 had a mean ± SD VEGF level of 454.44 ± 313.36 pg/mL pre-treatment and 456.71 ± 290.65 pg/mL post-treatment (p = 0.94). In CKD stage 3, the mean VEGF level was 618.35 ± 376.17 pg/mL before treatment and 592.22 ± 316.27 pg/mL after treatment, with no statistically significant difference (p = 0.498). These findings indicate that VEGF levels remained unchanged following autologous dendritic cell therapy across all subgroups (Table 3).

Table 3. Serum VEGF Level. Data are presented as the median ± interquartile range. Wilcoxon signed rank was used to calculate the p-value.

	Subject Group	VEGF Pre-Autologous Dendritic Cells (pg/mL)	VEGF Post-Autologous Dendritic Cells (pg/mL)	*p*-Value
	Overall (median ± IQR)	522.10 ± 608.6	473.70 ± 550	0.589
UACR	Microalbuminuria (median ± IQR)	305.05 ± 634.82	318.10 ± 533.32	0.913
	Macroalbuminuria (mean ± SD)	611.72 ± 319.10	604.35 ± 295.19	0.863
Stage CKD	CKD stages 1 and 2 (mean ± SD)	454.44 ± 313.36	456.71 ± 290.65	0.94
	CKD stage 3 (mean ± SD)	618.35 ± 376.17	592.22 ± 316.27	0.498

Our previous study has revealed that administering autologous dendritic cells can significantly reduce UACR in DKD patients. Decreases in TNF-α, a potent driver of inflammation, were also found after administration of

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autologous dendritic cells, which strongly suggests the cells can induce inflammation controls that eventually lead to improvement of UACR [12].

Endothelin levels were measured before and four weeks after autologous dendritic cell administration. In the overall analysis, the median endothelin level was 1.74 ± 0.71 pg/mL pre-treatment, slightly decreasing to 1.63 ± 0.76 pg/mL post-treatment. However, this change was not statistically significant ($p = 0.554$).

Subgroup analysis based on urinary albumin-to-creatinine ratio (UACR) categories showed that in the microalbuminuria group, the mean ± standard deviation (SD) endothelin level was 1.70 ± 0.47 pg/mL before treatment, decreasing slightly to 1.65 ± 0.60 pg/mL post-treatment ($p = 0.570$). In the macroalbuminuria group, the pre-treatment mean ± SD endothelin level was 1.91 ± 0.43 pg/mL, with a slight post-treatment decrease to 1.89 ± 0.47 pg/mL, which was not statistically significant ($p = 0.847$).

When stratified by CKD stage, patients with CKD stages 1 and 2 had a mean ± SD endothelin level of 1.62 ± 0.36 pg/mL pre-treatment, which slightly decreased to 1.59 ± 0.38 pg/mL post-treatment ($p = 0.604$). In CKD stage 3, the mean ± SD endothelin level was 1.93 ± 0.49 pg/mL before treatment, decreasing slightly to 1.89 ± 0.64 pg/mL post-treatment ($p = 0.712$). These results indicate that endothelin levels remained unchanged following autologous dendritic cell therapy across all subgroups (Table 4).

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Table 4. Serum endothelin level. Data are presented as the median ± interquartile range. Wilcoxon signed rank was used to calculate the p-value.

		Endothelin Pre-Autologous Dendritic Cells (pg/mL)	Endothelin Post Autologous Dendritic Cells (pg/mL)	*p*-Value
General (median ± IQR)		1.74 ± 0.71	1.63 ± 0.76	0.554
UACR	Microalbuminuria (mean ± SD)	1.70 ± 0.47	1.65 ± 0.60	0.57
	Macroalbuminuria (mean ± SD)	1.91 ± 0.43	1.89 ± 0.47	0.847
Stage CKD	CKD stages 1 and 2 (mean ± SD)	1.62 ± 0.36	1.59 ± 0.38	0.604
	CKD stage 3 (mean ± SD)	1.93 ± 0.49	1.89 ± 0.64	0.712

4. Discussion

In this study, we found that after autologous dendritic cell administration, the RI change was insignificant [from 0.74 ± 0.07 to 0.75 ± 0.06 ($p = 0.17$)]. RI is an indicator of renal resistance to perfusion and reflects arterial resistance. The normal range of RI values for the kidney is between 0.47 and 0.70, with the difference between the right and left kidneys not exceeding 5–8%. This value may increase with age or in renal disease conditions [15]. The resistive index reflects the differences between maximum and minimum blood flow velocities and the complex interaction between systemic circulation and renal microcirculation. An increase in RI indicates greater arterial stiffness and elevated pulsatility [15]. Based on several previous studies, changes in RI following revascularization occur within approximately 3 months [16].

However, administering autologous dendritic cells significantly reduced the median PI value from 1.61 ± 0.63 to 1.21 ± 0.26 ($p < 0.001$). This decrease indicates a significant change in blood flow dynamics or vascular resistance. Microangiopathy complications and renal dysfunction can be predicted by measuring the PI value with Doppler ultrasound. The decrease in PI observed in this study indicates a significant change in blood flow dynamics or vascular resistance, as an elevated PI value can predict kidney damage and increased vascular resistance associated with higher protein leakage into the urine. Likewise, lower PI values are generally associated with improved blood flow and reduced resistance within the vascular system [17]. The statistically significant decrease in PI following this therapy indicates that autologous dendritic cell has the

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potential as an adjunctive treatment to improve renal blood flow and reduce vascular resistance in patients with CKD [18]. This suggests that the albuminuria-lowering effect of autologous dendritic cell therapy was mediated by decreased vascular resistance in the kidney.

On the other hand, the administration of autologous dendritic cells had a similar effect on perfusion biomarkers VEGF and endothelin. The median VEGF value decreased from 522.10 ± 608.6 to 473.70 ± 550 ($p = 0.589$), and the mean endothelin value decreased from 1.79 ± 0.46 to 1.75 ± 0.55 ($p = 0.554$). However, these changes were not statistically significant. This is a positive impact of autologous dendritic cell administration on vascular regulation, potentially reducing the risk of vascular complications. The reduction in endothelin and VEGF suggests that autologous dendritic cells may have a beneficial modulatory effect on endothelial function. Previous studies have revealed that endothelin acts as a potent vasoconstrictor and is associated with increased blood pressure and cardiovascular risk [18]. Meanwhile, VEGF plays a significant role in the pathophysiology of DKD. In kidneys, VEGF-A is primarily expressed by podocytes and affects the structure and function of glomerular endothelial cells (GECs) by binding to its receptor, VEGFR-2. In early DKD, increased VEGF-A expression leads to glomerular damage, promoting proteinuria and endothelial dysfunction. While VEGF-A signaling can stimulate new blood vessel formation, its overactivation contributes to kidney damage, and the inhibition of VEGF in diabetic models has been shown to reduce proteinuria and mitigate glomerular injury [8]. Reducing endothelin levels may significantly impact renal function, particularly CKD. Endothelin-1 (ET-1) is a potent vasoconstrictor significantly affecting renal blood flow. Its overexpression can lead to decreased GFR, increased proteinuria, and podocyte damage. This primarily occurs through the activation of endothelin type A (ETA) receptors, which trigger vasoconstriction of afferent arterioles, hyperfiltration, and eventually expedited kidney damage [19,20]. Reducing endothelin levels may enhance GFR by decreasing vascular resistance in the kidneys [21,22]. However, despite a significant decrease in PI, non-significant changes in these markers suggest that other factors might mediate decreased vascular resistance.

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The pathogenesis of DKD involves multiple factors, such as chronic inflammation, endothelial dysfunction, fibrosis, and changes in renal perfusion. Studies have found that the administration of autologous DC can decrease UACR [12]. In this study, we studied renal perfusion and angiogenesis parameters to elucidate the mechanism of action on how autologous DC can decrease UACR. Although administering autologous dendritic cells may affect other aspects of renal or circulatory health, its impact on RI, VEGF, and endothelin was not statistically significant. However, it should be considered that autologous dendritic cells may require a longer duration to influence the regulation of RI, VEGF, and endothelin or that the reparative mechanisms induced by these cells are more complex and do not directly affect RI, VEGF, and endothelin significantly within the context of this study. Changes in RI and PI may evolve over a more extended period—potentially up to 3 months or more—which may partially explain the non-significant findings observed in the present study, which was designed as a short-term exploratory analysis. Nonetheless, the significant change observed in PI in this cohort provides a promising signal. It supports the rationale for conducting future studies with more extended follow-up periods to better assess the sustained effects and clinical relevance of dendritic cell therapy in DKD. Additionally, further research with a larger sample size is needed to explore other factors that may influence the response to autologous dendritic cells and their impact on managing vascular-related clinical conditions and understand the long-term effects of this therapy on CKD patients.

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5. Conclusions

This study evaluates the effects of autologous dendritic cell therapy on kidney perfusion in patients with DKD, focusing on changes in vascular markers. The results demonstrated a significant decrease in PI, indicating improved kidney perfusion. At the same time, changes in the RI, VEGF, and endothelin levels were not statistically significant. These findings suggest that the therapeutic mechanism of autologous dendritic cell therapy in DKD may involve the modulation of renal perfusion.

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Diffusion Tensor Imaging Magnetic Resonance Imaging Assessment in a Clinical Trial of Autologous Dendritic Cell Transfer for Diabetic Kidney Disease: A Molecular Approach

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1. Introduction

Diabetes mellitus (DM) global prevalence is estimated at 9.3%, with projections indicating an increase to 10.2% by 2030 and 10.9% by 2045 [1]. Among individuals with type DM, diabetic kidney disease (DKD) is present in about 27% of cases, and those affected by DKD have a mortality rate 18.3 times greater than those without diabetes [2].

Inflammation is a crucial factor in the pathophysiology DKD, where immune cell activation leads to the production of pro-inflammatory cytokines. Key biomarkers, including matrix metalloproteinase 9 (MMP-9) and intercellular adhesion molecule (ICAM-1), are involved in driving kidney damage through inflammatory processes and fibrosis [3,4,5]. While these biomarkers are well established in the disease process, their direct correlation with non-invasive imaging biomarkers, particularly in the context of immunotherapy, remains underexplored

Diffusion tensor imaging (DTI) has become a valuable non-invasive method for evaluating kidney microstructure, offering insights into tissue integrity by measuring mean diffusivity (MD) and fractional anisotropy (FA) [6]. DTI has gained recognition as a valuable imaging technique for assessing kidney inflammation and fibrosis by identifying structural changes at the microstructural level. DTI utilizes the diffusion properties of water molecules to map tissue microstructure, with FA serving as a key metric. FA has shown sensitivity to a variety of kidney diseases, including chronic kidney disease (CKD) and glomerulonephritis, by detecting subtle alterations in kidney tissue integrity [6,7]. Recent studies have highlighted the growing importance of DTI in evaluating early-stage kidney damage and monitoring the progression of renal diseases, particularly in the context of DKD [8]. FA values correlate closely with renal function and pathological changes, offering a promising method for monitoring CKD progression [8].

Autologous dendritic cells, derived from the patient's own immune system, have gained attention as a promising therapeutic option in modulating the immune response in various inflammatory conditions [9,10,11,12]. These cells can be genetically modified outside the body to enhance their functions on immune-regulatory before being reintroduced

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into the patient's system. This process helps promote immune tolerance and decrease inflammation [13]. The use of autologous dendritic cells in therapy has shown considerable promise in reducing inflammation and fibrosis in various medical conditions, making them a highly potential treatment strategy for managing diabetic kidney disease (DKD).

Despite the potential of using autologous dendritic cells to modulate inflammation in diabetic kidney disease (DKD), and the integration of DTI MRI for monitoring, this approach has not been thoroughly explored. This study seeks to address this gap by assessing the potency of autologous dendritic cell transfer on renal inflammation and fibrosis, utilizing DTI MRI and inflammatory biomarkers (MMP-9 and ICAM-1) as key indicators of therapeutic efficacy.

2. Materials and Methods

2.1. Study Design

This investigation utilized a quasi-experimental design, specifically employing a one-group pre-test post-test methodology. The study was carried out at the Army Central Hospital, and ethical approval was obtained from the Ethics Committee of Gatot Soebroto Army Central Hospital (Ethical Clearance No. 110/VIII/KEPK/2024, dated 23 August 2024). All participants provided written informed consent prior to their involvement. The clinical trial is registered with ClinicalTrials.gov under the registration number NCT06866158, initially submitted on 22 February 2025. During the preparation of this manuscript, the authors utilized ChatGPT 4o for language enhancement and proofreading purposes. However, the authors have thoroughly examined and refined the output, taking full responsibility for the accuracy and integrity of the content presented in this publication.

2.2. Research Subject

The study participants were patients diagnosed with diabetic kidney disease (DKD) from the internal medicine polyclinic at the Army Hospital. A nonprobability sampling method was utilized for participant selection. Figure 1 demonstrates the subject selection process. The initial pool

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comprised 10,930 patients from the internal medicine polyclinic. After narrowing down the selection, 1280 patients were from the Endocrine Clinic, and 312 were from the Kidney Clinic. A total of 33 patients expressed interest in participating, but seven were excluded, leaving 25 patients who met the inclusion criteria and proceeded with the study.

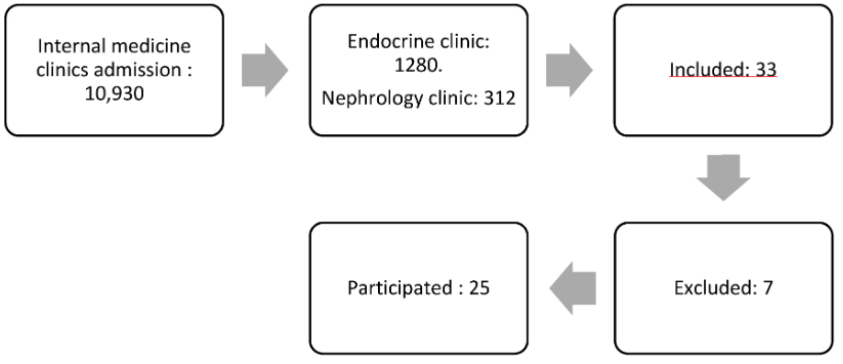

Figure 1. The flow of subject selection.

Patients were excluded from the study if they had undergone immunosuppressive treatment within the last four weeks, had other kidney diseases, were diagnosed with conditions causing proteinuria, had other types of DM, were pregnant, required oxygen supplementation, were undergoing cancer hormone therapy, history of thromboembolism, obesity with a BMI exceeding 40 kg/m2, or uncontrolled hypertension (systolic > 180 mmHg or diastolic > 100 mmHg).

The sample size for this study was determined utilizing the G*Power version 3.1.9.7 software, applying a two-tailed t-test approach. The calculation considered an effect size of 0.8, a significance level (α) of 0.05, and a statistical power of 0.95. Based on these parameters, the minimum required sample size was determined to be 23 participants to ensure sufficient statistical strength.

2.3. Research Procedure

The study procedure included several key steps. Initially, subjects were prepared for baseline measurements, which involved blood sampling to assess the biomarkers MMP-9 and ICAM-1, as well as the generation of

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autologous dendritic cells. Following this, subjects underwent Diffusion Tensor Imaging (DTI) MRI examinations of the kidneys.

The MMP-9, ICAM-1, and DTI MRI examinations were performed at two time points: before the transfer of autologous dendritic cells and four weeks following the treatment.

2.4. Study Product Generation

At the baseline of the study, blood samples were drawn from each participant at baseline. A total of 40 cc of whole blood was carefully processed and cultured with a specific culture medium enriched with Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Interleukin-4 (IL-4) was used for a period of five days. This culture process facilitated the differentiation of the peripheral blood mononuclear cells into dendritic cells. Following the differentiation phase, the dendritic cells were further incubated for an additional two days to promote their maturation. Upon achieving the desired level of maturation, the autologous dendritic cells were then transfered through injection, subcutaneoulsy into the participant's arm.

2.5. Laboratory Testing and MRI DTI

The biomarkers MMP-9 and ICAM-1 were quantified utilizing a sandwich enzyme-linked immunosorbent assay (ELISA) kit (Reed Biotech Ltd., Wuhan, China). For the DTI MRI imaging analysis, a MAGNETOM Vida MRI scanner (Siemens, Erlangen, Germany) was utilized in this study. DTI MRI examination was performed on both kidneys of the subject. Then the subject was subjected to 3 ROIs in each kidney. The three ROIs in each kidney were selected to represent the kidney's upper, middle, and lower poles, respectively. These ROIs were chosen by a single highly trained technician under the supervision of a senior radiologist. The technician was unaware of the ROI selection results before the intervention when selecting the ROIs for the DTI MRI after the intervention, ensuring unbiased selection. After obtaining the FA value. In this study, FA was taken, with a decrease and increase in FA in patients.

MRI Scanner Specifications as follows, the MAGNETOM Vida magnetic resonance imaging (MRI) scanner, identified by serial number 176241 and

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material code 11060815, was utilized in this study. Operating at a magnetic field strength of 3 Tesla (which is typical for MAGNETOM Vida models), the scanner was configured for high-resolution imaging. Imaging parameters included a field of view (FoV) with a reading FoV of 250 mm and a phase FoV of 81.3%. Thirty slices were acquired with each slice having a thickness of 1.8 mm. The repetition time (RT) was set to 5800 ms, and the echo time (ET) was 71 ms, with the phase encoding direction set to horizontal (H) to forward (F). Furthermore, phase oversampling was applied at 50%.

For the DTI analysis, multi-directional diffusion weighting (MDDW) was employed with a monopolar diffusion scheme across six distinct directions. The b-values used were b = 0 s/mm2 with five averages and b = 330 s/mm2 with twelve averages. The slice thickness remained consistent at 1.8 mm, while the ET and RT were maintained at 71 ms and 5800 ms, respectively. The imaging coil elements employed were of type SP2-4 to ensure optimal data acquisition.

Post-processing involved tensor-based analysis to generate FA maps, incorporating active noise correction, dynamic field correction, and noise masking to improve the accuracy of the results.

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2.6. Statistics

Normality was tested using Shapiro-Wilk for samples <50 and Kolmogorov-Smirnov for samples >50. FA DTI variables were analyzed with a paired t-test, and non-normal variables with the Wilcoxon signed-rank test. Linear regression assessed the impact of autologous dendritic cell transfer on MMP-9 and TGF β.

3. Results

3.1. Subject Characteristics

Table 1 presents the study participants' characteristic. Twenty five subjects who met the inclusion and exclusion criteria participated in the study and adhered to the research procedures throughout all stages. The participants had an average age of 63 years, ranging from 50 to 78 years. The group included 10 men and 15 women, with 13 diagnosed with microalbuminuria and 12 with macroalbuminuria. Hypertension was the most prevalent comorbidity, affecting 24 participants (96%).

Table 1. Subject's Characteristics.

Age	<60	9	36.0%
	≥60	16	64.0%
Gender	Women	15	60.0%
	Men	10	40.0%
UACR	Microalbuminuria	13	52.0%
	Macroalbuminuria	12	48.0%
Chronic Kidney Disease Stage	1	9	36.0%
	2	4	16.0%
	3	12	48.0%
Hypertension	No	1	4.0%
	Yes	24	96.0%
Neuropathy	No	8	32.0%
	Yes	17	68.0%
Heart Disease	No	14	56.0%
	Yes	11	44.0%
Stroke	No	23	92.0%
	Yes	2	8.0%
Insulin	No	7	28.0%
	Yes	18	72.0%
Sulphonylurea	No	19	76.0%
	Yes	6	24.0%

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Biguanide	No	21	84.0%
	Yes	4	16.0%
Thiazolidinedione (glitazone)	No	24	96.0%
	Yes	1	4.0%
Glinid	No	25	100.0%
	Yes	0	0.0%
SGLT2-i (glifozin)	No	17	68.0%
	Yes	8	32.0%
GLP-1 agonist	No	25	100.0%
	Yes	0	0.0%
DPP4-i (gliptin)	No	20	80.0%
	Yes	5	20.0%
α-glucosidase-i	No	23	92.0%
	Yes	2	8.0%
Central a-agonist (clonidine)	No	24	96.0%
	Yes	1	4.0%
Diuretic	No	22	88.0%
	HCT	3	12.0%
	Spironolactone	0	0.0%
a-blocker	No	25	100.0%
	Yes	0	0.0%
CCB	No	8	32.0%
	DHP	13	52.0%
	Non-DHP	4	16.0%
ACE-i	No	22	88.0%
	Yes	3	12.0%
b-blocker	No	15	60.0%
	Yes	10	40.0%

Abbreviations: ACE-i: Angiotensin Converting Enzyme Inhibitor, GLP-1: Glucagon-Like Peptide-1, SGLT2-i: Sodium-glucose Cotransporter-2 Inhibitor, HCT: Hydrochlorothiazide, CCB: Calcium Channel Blocker, DPP4-i: Dipeptidyl Peptidase-4 Inhibitor, UACR: Urinary Albumin-Creatinine Ratio, DHP: Dihydropyridine.

Additionally, the majority of participants were classified as overweight, with 11 subjects (44%) falling into this category based on their body mass index (BMI). The majority of participants (48%) were in stage 3 of chronic kidney disease (CKD), with 12 subjects diagnosed at this level.

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3.2. MRI Analysis of DTI, MMP-9, and ICAM-1

Table 2 shows the analysis of FA, MMP-9, and ICAM-1. In DTI MRI measurements (FA) before and after transfer of autologous dendritic cells (Figure 2), the median value before transfer was 242.57 ± 63.97. After transfer of autologous dendritic cells, the median increased to 305.61 ± 152.32. Statistical tests showed a significant difference with a p-value = 0.042. Figure 3a shows box and whisker FA on DTI MRI.

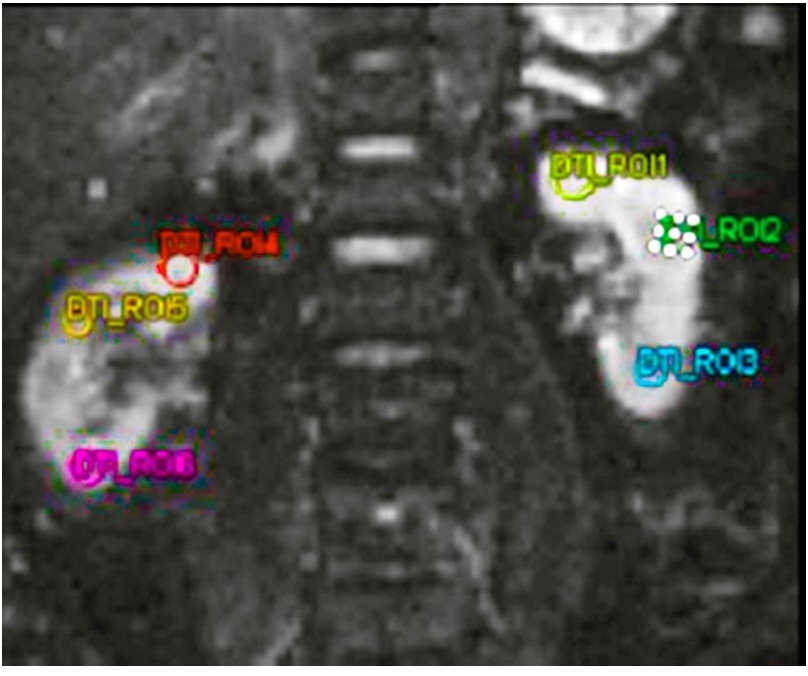

Figure 2. MRI DTI examination on a patient using 6 different region of interests. Abbreviations: MRI-DTI: Magnetic Resonance Imaging-Diffusion Tensor Imaging

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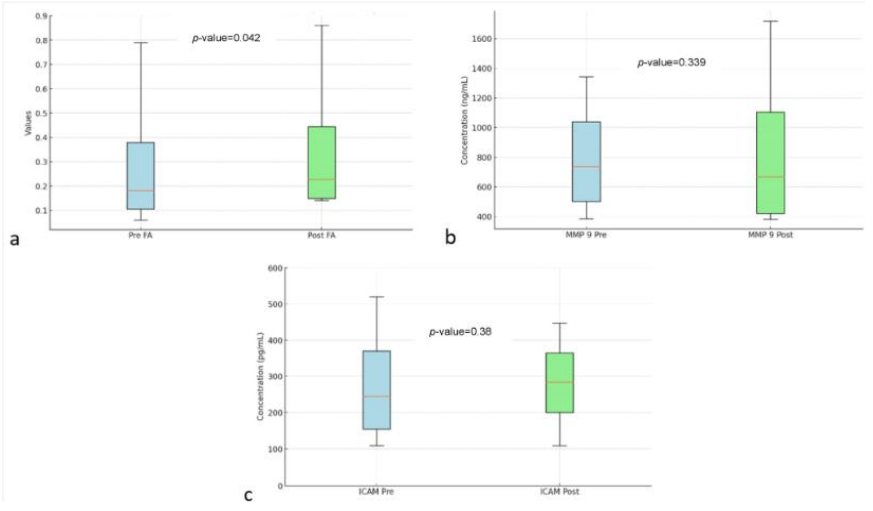

Figure 3. Box and whisker FA (a) MMP-9 (b) ICAM (c) before and after autologous dendritic cell transfer. Abbreviations: FA: Fractional Anisotropy, MMP-9: Matrix Metallopeptidase 9, ICAM: Intercellular Adhesion Molecule.

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Table 2. Analysis of FA, MMP-9, and ICAM before and after transfer of autologous dendritic cells.

		Before Autologous Dendritic Cells	After Autologous Dendritic Cell	*p*-Value
MRI DTI	FA (Median ± IQR)	0.242 ± 0.063	0.305 ± 0.152	0.042
Biomarkers	MMP-9 (Mean ± SD) ng/mL	873.68 ± 306.32	921.97 ± 333.33	0.339
	ICAM (Mean ± SD) pg/mL	321.74 ± 84.83	331.8 ± 61.05	0.38

Abbreviations: MRI DTI: Magnetic Resonance Imaging Diffusion Tensor Imaging, FA: Fractional Anisotropy, IQR: Interquartile Range, MMP-9: Matrix Metallopeptidase 9, SD: Standard Deviation, ICAM: Intercellular Adhesion Molecule.

Figure 3b shows the box and whisker MMP-9. MMP-9 biomarker, the mean value before intervention was 873.68 ± 306.32. After the intervention, the mean value increased to 921.97 ± 333.33. However, no statistically significant difference was found with a p-value = 0.339.

Figure 3c shows box and whisker ICAM-1. ICAM-1 biomarker, the mean before intervention was 321.74 ± 84.83 with a median of 320.2 ± 109.9. After transfer of autologous dendritic cells, the mean increased to 331.8 ± 61.05. However, no statistically significant difference was found with a p-value = 0.38.

Table 3 illustrates the relationships between the study variables. A significant negative correlation was observed between FA and MMP-9, with a correlation coefficient (r) of −0.347 and a p-value of 0.016. This suggests that FA increase is associated with a reduction in MMP-9 levels. In contrast, the relationship between FA and ICAM-1 was statistically not significant, with a p-value of 0.402. Similarly, no significant correlation was found between MMP-9 and ICAM-1, with a p-value of 0.409.

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Table 3. Relationship between variables in the study.

		Before Autologous Dendritic Cells	After Autologous Dendritic Cell	*p*-Value
MRI DTI	FA (Median ± IQR)	0.242 ± 0.063	0.305 ± 0.152	0.042
Biomarkers	MMP-9 (Mean ± SD) ng/mL	873.68 ± 306.32	921.97 ± 333.33	0.339
	ICAM (Mean ± SD) pg/mL	321.74 ± 84.83	331.8 ± 61.05	0.38

Abbreviations: MRI DTI: Magnetic Resonance Imaging Diffusion Tensor Imaging, FA: Fractional Anisotropy, IQR: Interquartile Range, MMP-9: Matrix Metallopeptidase 9, SD: Standard Deviation, ICAM: Intercellular Adhesion Molecule.

4. Discussion

This study revealed that hypertension was the most prevalent comorbidity among the research participants. Hypertension plays a role in exacerbating DKD by increasing intraglomerular pressure. DM and hypertension cause synergistic effects that increase endoplasmic reticulum stress and mitochondrial dysfunction in glomerular cells [14]. DKD associated with hypertension results from a chronic inflammatory reaction, which causes glomerular capillary injury and decreased renal function. This chronic inflammation will release pro-inflammatory cytokines, contributing to increased blood pressure and fibrosis in the kidney [15].

Based on the research results presented above, it can be seen that therapy using autologous dendritic cells has different effects on the various parameters measured. On MRI DTI FA, there was a significant improvement after therapy with a p-value of 0.042, indicating a positive change in tissue structure or function measured through MRI DTI FA imaging. This indicates that the therapy may have contributed to the improvement. FA in the kidney is related to the use of DTI sequences in renal imaging, which aims to assess the diffusion of water molecules in kidney tissue. FA is one of the important parameters in DTI that measures the direction and extent of water diffusion anisotropy, which is related to kidney structure and function. The use of DTI MRI in the kidney has shown that FA can be used as an indicator of microstructural damage, such as fibrosis or changes in the renal tubules. In the context of Diabetic Kidney Disease, FA measurement could be an important diagnostic tool to

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detect microstructural changes that cannot be seen through conventional imaging, enabling earlier detection and assessment of response to therapy [16]. FA values exhibit a negative correlation with serum creatinine and cystatin C, while showing a positive correlation with eGFR [7].

The underlying mechanism through which autologous dendritic cell transfer improves FA in DKD patients involves immune modulation, inflammation reduction, and potential kidney tissue repair. Dendritic cells, essential for immune responses, can be genetically modified to boost their immune-regulatory functions, thereby fostering tolerance and mitigating inflammation [13]. In DKD, chronic inflammation plays a significant role in kidney damage, contributing to fibrosis, endothelial dysfunction, and tubular injury [15]. By modulating the immune response, dendritic cells help reduce this inflammatory state, leading to improvements in kidney tissue integrity, as reflected by the increase in FA [6]. FA measures the integrity of kidney microstructure, with higher values indicating better tissue organization and function [7]. This improvement suggests that dendritic cell therapy may aid in kidney tissue repair by reducing fibrosis, enhancing podocyte function, and restoring the alignment of renal microstructures [17]. Ultimately, the increase in FA observed after dendritic cell transfer likely reflects the regenerative effects of the therapy, which promotes kidney repair through immune modulation and inflammation reduction.

This study also found a significant correlation between FA and inflammatory biomarker MMP-9 ($p = 0.025$). This finding is consistent with the study by Seah et al., which identifies a relationship between FA and inflammatory biomarkers [18]. In the early stages of DKD, MMP-9 increases as an initial response to hyperglycemia and oxidative stress. Increased MMP-9 is involved in tubular basement membrane degradation and inflammatory cell recruitment, leading to damage to the kidneys [19]. This study shows that an increase in FA is associated with a decrease in MMP-9. A decrease in MMP-9 can improve kidney function in DKD. Decreased MMP-9 can modulate podocyte function and inhibit dedifferentiation. Suggesting that lower MMP-9 may contribute to improved renal function by maintaining podocyte integrity and function, which is important in maintaining glomerular filtration [20]. MMP-9 is

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also positively associated with albuminuria, so a decrease in MMP-9 indicates a decrease in albuminuria [20].

In this study, there were changes in MMP-9 values that were not significant. Changes in MMP-9 are likely due to the longer follow-up time required in this study and the multiple transfer of autologous dendritic cells. This is consistent with the study by Miyazaki et al., which examined the changes in MMP-9 levels, noting a significant decrease within 12 months following the administration of ACE inhibitors (ACEi) or angiotensin II receptor blockers (ARB) during the same period [21].

This study found no significant alterations in ICAM-1 values. Future studies are expected to assess ICAM-1 once a week for one month. Research conducted by Shukla et al. assessed ICAM-1 seven days after the intervention [22]. Further research is expected to assess ICAM-1 once a week for seven days to assess changes in ICAM-1.

In this study, there were significant changes in FA on DTI MRI, while the biomarkers MMP-9 and ICAM-1 showed no significant changes. This may be because there are other inflammatory biomarkers, which can affect the value of FA. Research conducted by Tripathi et al. showed a significant correlation of FA with IL-1β and TNF α [23]. In research conducted by Rodrigue et al., there is a significant relationship between FA with IL 8 and TNF α [24].

5. Conclusions

Autologous dendritic cells transfer significantly enhanced FA in kidney tissue, suggesting its potential to improve kidney function in DKD. However, no significant changes in inflammatory biomarkers like MMP-9 and ICAM-1 underscores the complexity of immune modulation in DKD, indicating that multiple therapeutic mechanisms may be involved. While the results are promising, additional research with a larger sample size, randomized control groups, and extended follow-up periods is crucial to further validate the efficacy of dendritic cell therapy. Future studies should also investigate the potential synergistic effects of combining dendritic cell therapy with other treatments to optimize therapeutic outcomes. Moreover, expanding the range of biomarkers and imaging modalities, such as DTI with molecular imaging, could offer a more thorough understanding of the treatment's impact on kidney structure and function.

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Clinical Trial of Autologous Dendritic Cell Administration Effect on Water Molecule Diffusion and Anti-Inflammatory Biomarkers in Diabetic Kidney Disease

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1. Introduction

Diabetic kidney disease (DKD) is a severe microvascular complication in people with diabetes, which can develop into chronic kidney disease (CKD). Based on Global Burden of Disease data, approximately 27% of DM patients experience DKD, which contributes to 40% of total CKD cases [1]. In Indonesia, DKD is the second largest cause of stage 5 CKD after hypertension, with 28% of CKD patients exhibiting DKD [2]. DKD significantly increases the risk of death. Patients have a four times greater risk of dying within ten years than those without DKD [3]. In addition, the management of DKD imposes a high health cost burden, with approximately 10% of the total catastrophic disease costs in Indonesia allocated to the management of chronic kidney disease in 2021 [4].

Chronic inflammation in diabetes plays a vital role in the development of DKD. This inflammation releases reactive oxygen species (ROS) and inflammatory mediators that damage kidney tissue, triggering fibrosis and changes in kidney structure [5]. The adhesion molecule Intercellular Adhesion Molecule-1 (ICAM-1) plays a role in the progression of DKD by facilitating leukocyte adhesion and infiltration into renal tissue, which in turn increases albuminuria and renal inflammation [6,7]. Studies show that ICAM-1 expression correlates with the degree of albuminuria in DKD patients, making it an essential indicator for diagnosis. Structural changes in the kidney due to fibrosis can also be identified radiologically through diffusion-weighted magnetic resonance imaging (DW-MRI). A decrease in the apparent diffusion coefficient (ADC) value produced by DW-MRI indicates microvascular damage in the kidney, which is helpful in detecting DKD early [8].

Although standard therapies such as renin–angiotensin system (RAS) inhibitors and sodium-glucose cotransporter 2 (SGLT2) inhibitors have been shown to slow the rate of progression of DKD and reduce the severity of microalbuminuria, the results are still unsatisfactory, especially in patients with normal blood pressure or without microalbuminuria. The combination of the two therapies has also not completely stopped the progression of DKD [9,10]. Therefore, there is an urgent need to develop additional therapies that can target the underlying pathogenesis of DKD,

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namely chronic low-grade inflammation. One potential therapy being investigated is the use of autologous dendritic cells. These dendritic cells, which are part of the innate immune system, can suppress the inflammatory response. Research suggests that autologous dendritic cells can be used as anti-inflammatory agents in various diseases, including arthritis, kidney disease, and autoimmune diseases [11,12,13].

Dendritic cells (DCs) can reduce inflammation through various mechanisms that promote immune tolerance and homeostasis. They can differentiate into tolerogenic dendritic cells (tolDCs), which suppress excessive immune responses and help the immune system recognize self-antigens, preventing autoimmune reactions. DCs also produce anti-inflammatory cytokines like interleukin (IL)-10 and transforming growth factor-β (TGF-β), which inhibit pro-inflammatory cells and promote the activation of regulatory T cells (Tregs) that further limit inflammation. Additionally, DCs resist maturation in the presence of anti-inflammatory signals, interact with other immune cells like NK cells and macrophages to fine-tune the immune response, and can be used in therapies to induce targeted immune responses without triggering widespread inflammation [14,15]. Autologous DCs have also shown potential for reducing inflammation in autoimmune diseases like Systemic Lupus Erythematosus (SLE), with a case study demonstrating significant clinical improvement after DC-based therapy. The treatment involved using DCs derived from the patient's blood and maturated to stimulate an immune response, leading to improvements in symptoms such as joint pain and muscle weakness [16]. With the potential for ex vivo generation of anti-inflammatory DCs, these cells offer promising therapeutic possibilities for treating inflammatory and autoimmune diseases, including diabetic kidney disease [17]. Dendritic cells' anti-inflammatory potential can be an effective adjuvant therapy to suppress inflammation in DKD, prevent further damage to kidney structure and function, and slow disease progression. This study aims to determine the effect of autologous dendritic cell administration in reducing inflammation in DKD and slowing disease progression, as measured by ADC, TGF-β, and ICAM-1 parameters.

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2. Materials and Methods

Study Design

This study is a quasi-experimental clinical trial with a pre-test and post-test design, which aims to evaluate changes in ADC in DKD patients after administration of autologous dendritic cells. This study was conducted at Gatot Soebroto Army Central Hospital (RSPAD GS). The Health Research Ethics Committee of RSPAD GS approved the study. Before the start of the study, all subjects were given clear information about the study's purpose, procedures, risks, and benefits. The subjects were allowed to ask questions, and after fully understanding, they signed an informed consent form as a sign of their agreement to participate in this study. Strict research ethics were applied to protect the subjects' rights during the clinical trial. The study subjects consisted of DKD patients who met the inclusion criteria and were outpatients at the polyclinic of RSPAD GS during April 2024. The sampling technique used a consecutive sampling method, where subjects who met the requirements would be included in the study sequentially until the minimum quota of 20 subjects was met. With this number of subjects, it is expected to detect significant changes in ADC values and substantial modifications in TGF-β and ICAM-1.

The inclusion criteria for this study required participants to be over 18 years old, able to understand and sign informed consent, and capable of complying with the research procedures. They also had to meet the 2021 PERKENI diagnostic criteria for Type 2 Diabetes Mellitus (DM), such as fasting plasma glucose of ≥126 mg/dL (normal < 126 mg/dL), blood glucose ≥200 mg/dL (normal < 200 mg/dL) two hours after a glucose tolerance test, or HbA1c ≥ 6.5% (normal: < 5.7, prediabetes: 5.7–6.4). Additionally, participants needed an estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m2 (normal ≥ 90 mL/min/1.73 m2) and a urine albumin-to-creatinine ratio (UACR) ≥30 mg/g (normal < 30 mg/g). Exclusion criteria included recent immunosuppressive treatment, kidney diseases like polycystic kidney disease or lupus nephritis, other proteinuria-causing conditions, a positive pregnancy test, immunodeficiency disorders (HIV, HCV, HBV), cancer under active treatment (except hormonal therapy), and thromboembolic history.

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Participants with physical or mental disabilities, uncontrolled hypertension (SBP > 170 mmHg, DBP >100 mmHg), or BMI over 40 kg/m2 were also excluded.

A 40-milliliter blood sample was collected from each participant, and Peripheral Blood Mononuclear Cells (PBMCs) were isolated using density gradient centrifugation with Ficoll-Paque. These PBMCs were then cultured with Granulocyte–Macrophage Colony-Stimulating Factor (GM-CSF) and interleukin-4 (IL-4) for five days to generate immature DCs. Afterward, the cells were incubated with an antigen for two additional days to induce maturation. The quantity of dendritic cells administered varied between participants based on the yield from their individual blood samples, with no adjustments to standardize cell counts. Consequently, the total dose administered ranged from approximately 0.5 to 8 million DCs per participant, depending on the specific yield from each sample.

In the first week, a screening phase was conducted to determine the eligibility of subject participation according to predetermined criteria. After that, subjects underwent a baseline examination, which included an MRI to assess ADC and initial laboratory tests. Blood was drawn to prepare autologous dendritic cells that would later be used in the injection process. After that, in the intervention phase, autologous dendritic cells were injected into each subject. After the injection, an evaluation of ADC was conducted in week 4. In addition, laboratory evaluation and inflammatory biomarkers, TGF-β and ICAM-1, were also performed at baseline and four weeks after injection to see changes in the inflammatory response. Both biomarkers were measured using sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits (Reed Biotech Ltd., Wuhan, China), known for their high sensitivity and specificity in detecting these proteins in serum.

ADC measurements on renal MRI were performed using ADC maps generated from DWI (diffusion-weighted imaging) at a value of b = 1000 s/mm2. The senior radiographer defined three ROIs (Regions of Interest) in each kidney in the parenchymal area without distinguishing between the cortex or medulla. Each ROI was analyzed to obtain the ADC value in mm2/s.

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The criteria for choosing between parametric and non-parametric analysis in this study were based on the distribution of the data. The Shapiro–Wilk normality test was initially performed to assess whether the data followed a normal distribution. If the data were found to be normally distributed, this study applied parametric analysis, specifically the Paired t-test, to compare the differences before and after the intervention. This test assumes that the data follow a normal distribution and that the differences between paired observations are symmetrically distributed.

If the data were found to be non-normally distributed (i.e., did not meet the assumptions for parametric testing), non-parametric analysis was used. In this case, the Wilcoxon Signed-Rank Test was employed to compare the before and after intervention data. The Wilcoxon test does not require the assumption of normality and is appropriate for ordinal or skewed data.

To assess relationships between continuous variables, Pearson correlation analysis was used when the data were normally distributed, as it assumes a linear relationship and normality between variables. If the data were non-normally distributed, Spearman's rank correlation was applied, as it is a non-parametric test that does not assume normality and is suited for ordinal or skewed data.

For subgroup analysis, if comparing two independent groups with normally distributed data, the Independent Samples t-test was used. If the data in the subgroups were non-normally distributed, the Mann–Whitney U test (a non-parametric equivalent of the Independent Samples t-test) was used to compare differences between the groups (Figure 1). Statistical analyses were done using IBM SPSS Statistics 25 (IBM, Armonk, NY, USA).

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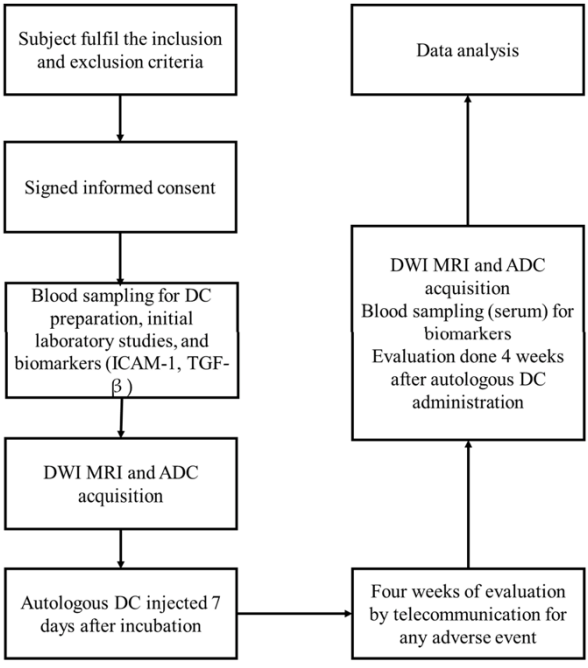

Figure 1. Study flowchart.

3. Results

3.1. Subject Characteristics

After screening based on the inclusion criteria, 156 patients met the requirements, while 510 patients did not. Of the 156 patients who met the criteria, 23 agreed to become research subjects, while 133 patients refused. Of the 23 consenting subjects, 22 underwent this study, but 1 was excluded due to meeting the exclusion criteria.

This study included 22 subjects who met the inclusion and exclusion criteria and completed the entire study procedure. The average age of the subjects was 64 years, with 9 (41%) men and 13 (59%) women. Hypertension was the most common comorbidity experienced by 20 subjects (90.9%). Most subjects had a body mass index in the overweight category, with nine subjects (40.9%). Most subjects also used insulin, as many as 17 subjects (77.3%) (Table 1).

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Table 1. Subjects' baseline characteristics.

Baseline Characteristics		
Number of subjects		22
Gender, n (%)	Men	9 (41)
	Women	13 (59)
Age, mean ± SD		64 ± 7.8
Comorbidities, n (%)	Hypertension	20 (90.9)
	Heart Disease	10 (45.5)
	Stroke	3 (13.6)
	Neuropathy	14 (63.6)
BMI categories, n (%)	Underweight (≤17.5 kg/m^2)	1 (4.5)
	Normal weight (17.50–22.99 kg/m^2)	6 (27.3)
	Overweight (23.00–27.99 kg/m^2)	9 (40.9)
	Obesity (≥28 kg/m^2)	6 (27.3)
Types of anti-diabetics consumed, n (%)	Sulphonylurea	6 (27.3)
	Biguanide	5 (22.7)
	Dipeptidyl peptidase-4 inhibitors	4 (18.2)
	Sodium-glucose cotransporter-2 inhibitors	8 (36.4)
	Insulin	17 (77.3)
Anti-hypertensive types consumed, n (%)	Angiotensin receptor blocker	15 (68.2)
	Angiotensin-converting enzyme inhibitors	3 (13.6)
	Calcium channel blockers	16 (72.7)
	Beta-blockers	9 (40.9)

3.2. Correlation of ADC and eGFR

The ADC values, representing the diffusion of water molecules within kidney tissue, were recorded from six ROIs (Regions of Interest) across the right and left kidneys (Figure 2). For the left kidney, pre-intervention

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ADC values ranged from 1.734 to 1.779 × 103 mm2/s across ROI 1 to ROI 3, while post-intervention values ranged from 1.529 to 1.736 × 103 mm2/s. A statistically significant reduction was observed in ROI 1 (p = 0.017), whereas no significant changes were found in ROI 2 (p = 0.910) or ROI 3 (p = 0.615). Similarly, in the right kidney, pre-intervention ADC values ranged from 1.818 to 1.951 × 103 mm2/s across ROI 4 to ROI 6. Post-intervention values showed slight decreases, ranging from 1.675 to 1.824 × 103 mm2/s. No statistically significant changes were observed in ROI 4 (p = 0.277), ROI 5 (p = 0.101), or ROI 6 (p = 0.709) (Table 2).

Table 2. Change in ADC on each ROI.

ADC Values ($\times 10^3$ mm^2/s)	Lef Kidney			Right Kidney		
	ROI 1	ROI 2	ROI 3	ROI 4	ROI 5	ROI 6
Pre	1.734	1.740	1.779	1.839	1.951	1.818
Post	1.529	1.728	1.736	1.675	1.824	1.773
p-value	0.017	0.910	0.615	0.277	0.101	0.709

Hypothesis test using Wilcoxon: ROI 1: left kidney, upper region; ROI 2: left kidney, middle region; ROI 3: left kidney, lower region; ROI 4: right kidney, upper region; ROI 5: right kidney, middle region; ROI 6: right kidney, lower region.

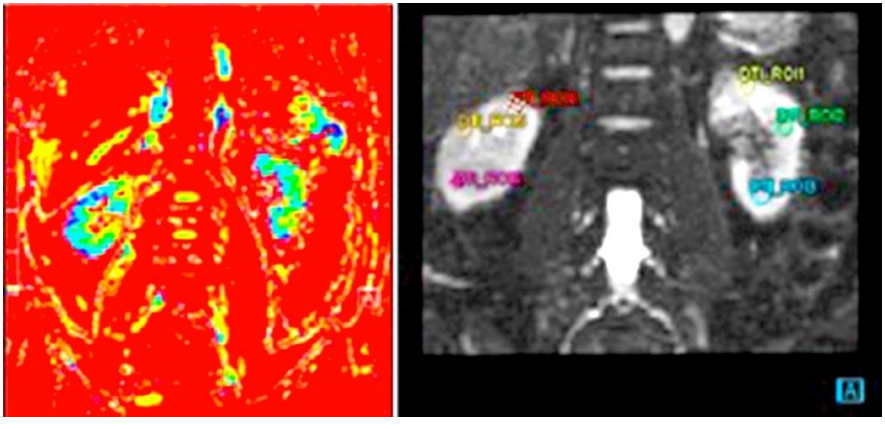

Figure 2. DWI image and ADC measurement on six different ROIs. A coronal DW-MRI and ADC map were obtained from a 68-year-old male volunteer with an eGFR of 70.65. Three ROIs were placed in the upper

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pole, middle portion, and lower pole of both kidneys. The mean ADC values for the left and right kidneys were 2.695 × 10−3 mm2/s and 2.877 × 10−3 mm2/s, respectively.

In the group with eGFR > 60 mL/min/1.73 m2, the correlation value between ADC and eGFR was −0.267, indicating a weak negative correlation between the two variables. However, the p-value of 0.457 indicates that this relationship is not statistically significant, so it cannot be concluded that there is a meaningful relationship between ADC and eGFR in this group (Table 3).

Table 3. ADC and eGFR correlation based on eGFR level.

	Correlation of ADC and eGFR	*p*-Value *
eGFR > 60 mL/min/1.73 m^2	−0.267	0.457
eGFR < 60 mL/min/1.73 m^2	0.079	0.806

* Correlation test using Spearman's rank correlation.

In contrast, in the group with eGFR < 60 mL/min/1.73 m2, the correlation value between ADC and eGFR was 0.079, indicating a very weak positive correlation between the two variables. The p-value of 0.806 also suggested that this relationship was not statistically significant. Therefore, there was no substantial evidence to suggest a meaningful relationship between ADC and eGFR in this group.

3.3. Changes in ADC

In this study, ADC values were measured before and after the intervention. The median ADC value before the intervention was 1.75 mm2/s, with a first to third quartile range (Q1–Q3) of 1.51 to 1.87 mm2/s. After the intervention, the median ADC decreased to 1.64 mm2/s, with a quartile range of 1.46 to 1.75 mm2/s. This decrease indicates a change in ADC values post intervention. The normality test using Shapiro–Wilk showed that the ADC data before the intervention were normally distributed with a p-value of 0.223. Furthermore, hypothesis testing using the Wilcoxon Signed Rank Test yielded a p-value of 0.003, indicating a statistically

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significant difference between ADC values before and after the intervention (Table 4, Figure 3).

Table 4. Change in ADC, ICAM-1, and TGF-β.

Variables	Median (Q1–Q3)	*p*-Value Hypothesis Test
ADC Pre	1.75 mm^2/s (1.51–1.87)	0.223 [a]
ADC Post	1.64 mm^2/s (1.46–1.75)	
ICAM-1 Pre	325.6 ng/mL (256–355.3)	0.359 [b]
ICAM-1 Post	336.7 (283.8–375.3)	
TGF-β Pre	39.55 ng/mL (30.9–49)	0.506 [a]
TGF-β Post	41.35 ng/mL (32.4–53.7)	

[a] Hypothesis testing using the Wilcoxon Signed Rank Test; [b] hypothesis testing using the Paired *t*-test.

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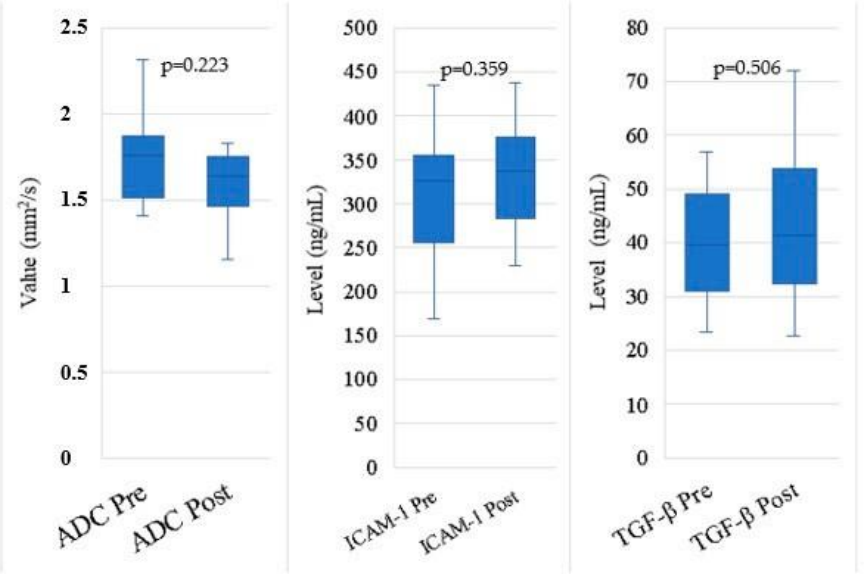

Figure 3. Change in ADC, ICAM-1, and TGF-β.

3.4. Changes in ICAM-1 and TGF-β Levels

For ICAM-1 parameters, the median value before autologous dendritic cell administration was 0.32 ng/mL with an IQR of 0.25–0.35, and these data were typically distributed based on the Shapiro–Wilk test (p = 0.668). Hypothesis testing using the Paired t-test showed no significant difference between pre- and post-administration values of autologous dendritic cells (p = 0.359). Post administration of autologous dendritic cells, the median ICAM-1 value increased to 0.33 ng/mL with an IQR of 0.28–0.37 and a normality test p-value of 0.798.

In the TGF-β parameter, the median value before autologous dendritic cell administration was 39.55 ng/mL with an IQR of 30.9–49. The normality test showed that the data were normally distributed (p = 0.36). Hypothesis testing using the Wilcoxon Signed Rank Test showed no significant difference between pre- and post-administration values of autologous dendritic cells (p = 0.506). After the administration of autologous dendritic cells, the median TGF-β value increased to 41.35 ng/mL with an IQR of 32.4–53.7 and a normality test p-value of 0.346.

3.5. Correlation of ADC, TGF-β, and VCAM

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Correlations involving ICAM-1 show weaker relationships, with coefficients such as 0.122 (ICAM-1 Pre with TGF-β Pre) and −0.004 (ICAM-1 Post with TGF-β Pre), both with non-significant p-values. The mean pre-ADC values indicate a significant correlation with ICAM-1 Pre (−0.384, p-value 0.039), while other mean ADC comparisons exhibit non-significant p-values (Table 5, Figure 4). Overall, the table summarizes the relationships between the variables, highlighting the varying strengths and significances of these correlations.

Table 5. ADC, ICAM-1, and TGF-β level correlation.

	TGF-β Pre	TGF-β Post	ICAM-1 Pre	ICAM-1 Post	Mean ADC Pre	Mean ADC Post
TGF-β Pre	1.000	0.778	0.122	−0.004	−0.108	0.040
p-value		0.000	0.294	0.493	0.316	0.430
TGF-β Post	0.778	1.000	0.010	−0.127	−0.029	0.176
p-value	0.000		0.483	0.287	0.449	0.217
ICAM-1 Pre	0.122	0.010	1.000	0.670	−0.384	−0.069
p-value	0.294	0.483		0.000	0.039	0.380
ICAM-1 Post	−0.004	−0.127	0.670	1.000	−0.086	−0.248
p-value	0.493	0.287	0.000		0.351	0.133
Mean ADC Pre	−0.108	−0.029	−0.384	−0.086	1.000	0.402
p-value	0.316	0.449	0.039	0.351		0.032
Mean ADC Post	0.040	0.176	−0.069	−0.248	0.402	1.000
p-value	0.430	0.217	0.380	0.133	0.032	

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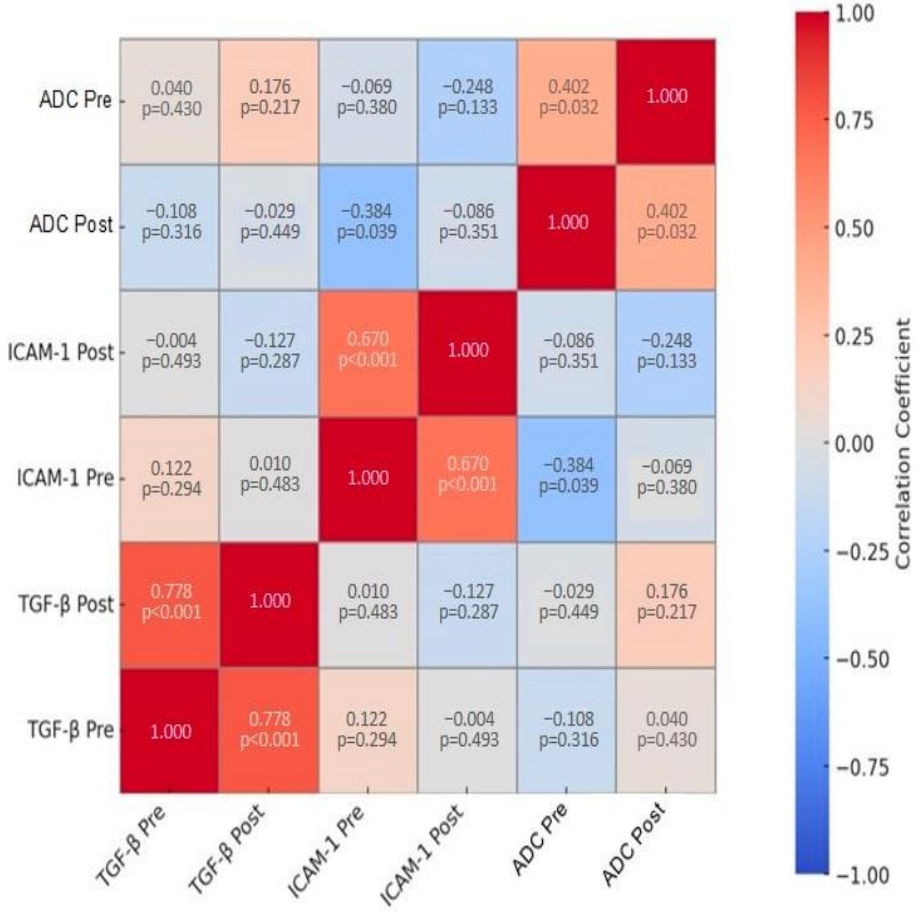

Figure 4. Heat map of variables' correlation coefficients.

Further analysis was performed to determine the effect of autologous dendritic cell administration on ADC values, TGF-β levels, and ICAM-1 by looking at the correlation between variables. Each variable was compared to the ratio of its value from post administration divided by the value pre-administration of autologous dendritic cells. The median of each variable ratio was determined. ADC, TGF-β, and ICAM-1 ratios above the median value were considered to have worsened, and vice versa, ratios below the median value were considered to have improved. Pearson's correlation test showed that the ICAM-1 ratio and ADC ratio had a significant negative correlation with $p = 0.010$. At the same time, the TGF-

β ratio and ADC ratio were positively correlated but not significant, with p-value = 0.479 (Table 6).

Table 6. ADC, ICAM-1, and TGF-β ratio correlation.

	ADC Ratio	
	Correlation	***p*-Value ***
ICAM-1 Ratio	−0.490	0.010
TGF-β ratio	−0.12	0.479

* Correlation test using Spearman's rank correlation.

3.6. Changes in ADC Values, Serum Creatinine Levels, ICAM-1, and TGF-β in Gender Subgroups

The mean change in ICAM-1 levels in men was 18.23 ± 55.1 with a p-value of 0.35, indicating that this change was not statistically significant ($p > 0.05$). On the other hand, the mean change in ICAM-1 levels in women was −31.77 ± 49.8 with a p-value of 0.04, indicating that this change was statistically significant ($p < 0.05$) (Table 7). These results suggest a significant difference in the shift in ICAM-1 levels in women after the intervention, while there was no significant change in men.

Table 7. ADC values, serum creatinine levels, ICAM-1, and TGF-β level changes in gender subgroups.

	Paired *t*-Test		**Wilcoxon Signed Rank Test**					
	Mean ICAM-1 (Post–Pre)	***p*-Value**	**Z TGF-β**	***p*-Value**	**Z ADC**	***p*-Value**	**Z Creatinine**	***p*-Value**
Men	−18.23 ± 55.1	0.35	−1.244	0.214	−0.533	0.594	−0.59	0.953
Women	31.77 ± 49.8	0.04	−0.105	0.917	−0.943	0.345	−2.030	0.042

Changes in TGF-β levels and ADC values before and after the administration of autologous dendritic cells in men and women showed no significant differences. In men, the p-value for changes in TGF-β was 0.214, and for ADC, it was 0.594, which indicated no significant change

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after autologous dendritic cell administration. Similarly, in women, the p-value for TGF-β was 0.917, and for ADC, it was 0.345, indicating no significant change in either variable after autologous dendritic cell administration. Creatinine levels in both groups also showed different results. Although the trends of both groups were similar, there was a decrease in creatinine levels after autologous dendritic cell administration. Still, there was a significant decrease in creatinine in the female group (Table 7).

3.7. Changes in ADC Values, Serum Creatinine Levels, ICAM-1, and TGF-β Based on Cardiovascular Comorbidities

Table 8 presents information focused on two specific groups. Those with cardiovascular comorbidities, including heart failure, coronary heart disease, and acute coronary syndrome, and those with resistant hypertension are defined as patients requiring three or more anti-hypertensive medications without achieving the target blood pressure. The results indicate that there were no significant differences in ADC, ICAM-1, TGF-β, or creatinine levels between patients with and without heart disease comorbidities at both pre-and post-autologous DC administration. Similarly, the analysis revealed no significant differences in these biomarkers between patients with and without resistant hypertension. The findings suggest that the presence of cardiovascular comorbidities and resistant hypertension does not appear to influence the outcomes of autologous DC administration in this patient population.

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Table 8. ADC values, serum creatinine levels, ICAM-1, and TGF-β changes in heart disease group and resistant hypertension group.

Variables	Time Point	Group					
		Heart Disease Comorbidity			Resistant Hypertension		
		Absent (*n* = 12)	Present (*n* = 10)		Absent (*n* = 14)	Present (*n* = 8)	
		Mean ± SD		*p*-Value [1]	Mean ± SD		*p*-Value [1]
ADC [a] (mm^2/s)	Pre	1.680 (0.229)	1.683 (0.891)	0.895	1.668 (0.381)	1.806 (0.611)	0.539
	Post	1.757 (0.288)	1.1517 (0.308)		1.637 (0.287)	1.664 (0.767)	
	p-value [2]	0.480	0.445		0.510	0.208	
ICAM-1 [b] (ng/mL)	Pre	329.97 ± 27.87	312.33 ± 19.44	0.578	338.25 ± 21.68	293.42 ± 27.53	0.300
	Post	334.94 ± 16.97	331.26 ± 16.23		339.89 ± 14.41	321.67 ± 19.96	
	p-value [2]	0.767	0.333		0.893	0.311	
TGF [b] (ng/mL)	Pre	44.67 ± 47.14	36.422 ± 28.41	0.465	45.47 ± 39.70	3.29 ± 2.56	0.502
	Post	45.51 ± 40.78	40.54 ± 43.75		46.67 ± 38.98	3.73 ± 3.87	
	p-value [2]	0.796	0.193		0.667	0.282	
Creatinine [b] (mg/dL)	Pre	1.27 ± 0.15	1.59 ± 0.17	0.544	1.56 ± 0.15	1.16 ± 0.17	0.468
	Post	1.24 ± 0.17	1.50 ± 0.14		1.52 ± 0.13	1.06 ± 0.19	
	p-value [2]	0.636	0.200		0.595	0.199	

[a] Result are presented as median (interquartile range), as the data were non-normally distributed; [b] the data were normally distributed; [1] Independent Samples *t*-test (for normally distributed data) or Mann–Whitney test (for non-normally distributed data) used to assess the difference between groups; [2] Paired *t*-test (for normally distributed data) or Wilcoxon (for non-normally distributed data) used to compare between pre- and post-autologous DC administration.

4. Discussion

This study showed that ADC values did not correlate with the severity of DKD. This result contrasts previous studies showing a significant correlation between mean renal ADC values and various degrees of severity of chronic kidney disease (CKD). Prior studies generally found that ADC values decreased as the severity of CKD increased (Çakmak et al., 2014a [8]). However, no similar pattern was found in this study. This study differentiated the degree of CKD based on grades 1–2 and 3. The lack of correlation between ADC and GFR is similar to the meta-analysis

by Liu et al., who explained that although ADC was able to differentiate between CKD grade 1–2 and normal kidney, there was no significant difference in ADC values between CKD grade 1–2 and grade 3. Liu et al. found significant heterogeneity when comparing ADC between grade 3 and grade 1–2 CKD, possibly due to variations in imaging parameters, ROI (Region of Interest) determination methods, and b values used in various studies. In addition, pathological changes in grade 1–2 CKD may still be similar to those in grade 3 CKD, such as fibrosis and decreased perfusion that have not led to significant differences in ADC values. This explains why the correlation between ADC and GFR is inconsistent, especially in the early to intermediate stages of CKD [18].

The results showed that autologous dendritic cell administration did not significantly change ADC, TGF-β, and ICAM-1 parameters. However, more detailed findings showed a dynamic relationship between TGF-β and ICAM-1 before and after autologous dendritic cell administration and a significant correlation between ICAM-1 and ADC in specific subgroups. Before the administration of autologous dendritic cells, it was found that TGF-β and ICAM-1 had a significant positive correlation. This indicates that in patients with DKD who have not received the intervention of autologous dendritic cell administration, TGF-β plays a vital role in changes in ICAM-1 levels. This positive correlation suggests that increased TGF-β levels are likely to be associated with increased ICAM-1 activity, which plays a role in the inflammatory process and fibrosis in the kidney. This was revealed by Chen et al. in that TGF-β is a regulator of the renal fibrosis process in DKD. TGF-β will promote excessive extracellular matrix accumulation through collagen and fibronectin production. Although not directly related to ICAM-1, TGF-β-induced fibrosis alters renal hemodynamics, increasing ICAM-1 expression [19]. However, TGF-β has a paradoxical role in inflammation, with its anti-inflammatory function. This is evidenced when the inhibition of TGF-β will increase inflammation [20]. Increased TGF-β1 plays a role in converting naive T cells into regulatory T cells (Treg), which can suppress excess immune responses and reduce inflammation, thus helping to repair kidney damage in diabetic kidney disease (DKD). In addition, TGF-β1 can reduce leukocyte and macrophage infiltration and suppress the production

of pro-inflammatory cytokines such as IL-1β and TNF-α, directly contributing to reducing inflammation and slowing the progression of DKD [21].

In line with these findings, a subgroup analysis based on gender found that the increase in ICAM-1 levels in female subjects was statistically significant. Research by Hwang et al. also explained that women or people with diabetes will have significantly higher ICAM-1 levels than men or people without diabetes [22]. A significant improvement also followed these results in serum creatinine levels. This finding might be caused by estrogen's protective role. Estrogen protects against hypertension by reducing sympathetic activity, enhancing baroreflex sensitivity, lowering oxidative stress, increasing nitric oxide production, and balancing the renin–angiotensin–aldosterone system. Through estrogen receptors in brain regions like the subfornical organ, paraventricular nucleus, and rostroventrolateral medulla, it modulates neurogenic and inflammatory pathways to lower blood pressure [23].

This finding is interesting because increased ICAM-1, usually associated with increased inflammation, is followed by improved renal function, reflected in decreased serum creatinine. This could reflect the fact that in the female subgroup, the response to autologous dendritic cell administration is different from that of men, where increased ICAM-1 levels may not only reflect increased adverse inflammation but also a more adaptive or regulative immune response to inflammation. This finding is also consistent with the study by Bui et al., who stated that, based on current evidence, ICAM-1 has a role in inflammation subsidence and tissue repair. This is achieved by modulating the efferocytosis function of macrophages for repair. Although there is no evidence of epithelial and endothelial tissue repair in the kidney, the effect of tissue healing in the colon, skin, and cornea hints at the possible influence of ICAM-1 on improving kidney function due to DKD [24]. Endothelial damage is a key component in the pathogenesis of DKD, contributing significantly to its progression. As part of the pathological features, endothelial cell injury occurs alongside glomerular mesangial expansion, basement membrane thickening, podocyte loss, and nodular glomerulosclerosis. This endothelial damage is not merely an incidental finding but plays a central

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role in the development of the disease [25]. In the early stages, it leads to tubular hypertrophy, which eventually progresses to interstitial fibrosis and tubular atrophy. This progression highlights the critical role of endothelial dysfunction in driving the pathological changes seen in DKD [26].

This study has several limitations that should be considered. The small sample size of 22 patients limits the generalizability of the findings, and the quasi-experimental design without a control group reduces the ability to draw firm conclusions on the treatment's efficacy. While changes in biomarkers like TGF-β and ICAM-1 were observed, no significant alterations in ADC values or kidney function were found, suggesting that more refined methods or longer follow-up may be necessary to detect meaningful effects. Additionally, the focus on gender differences and comorbidities indicates the need for larger, more diverse studies to validate these findings.

5. Conclusions

This study evaluated the impact of autologous dendritic cell administration on markers of kidney function and inflammation in diabetic kidney disease (DKD). The results showed no significant changes in ADC values, TGF-β levels, or overall kidney function across the patient cohort. However, a gender-specific response was observed, with significant changes in ICAM-1 levels in female participants, suggesting a possible adaptive immune response. This finding warrants further investigation into the role of gender in immune modulation and treatment response. While this study identified promising trends, such as potential improvements in renal function in females, the absence of significant overall changes in key biomarkers and kidney function suggests that the further optimization of treatment protocols is necessary.

Looking ahead, future research should aim to address the limitations of this study, including the small sample size and the lack of a control group, by conducting larger, randomized controlled trials. These trials should incorporate diverse patient populations, accounting for variables such as comorbidities, medication use, and baseline immune status, which may influence the effectiveness of dendritic cell therapy. Additionally,

exploring the long-term effects of dendritic cell administration and its potential for slowing disease progression in DKD is essential. Further studies should also investigate the mechanisms underlying the observed gender differences and explore potential combinatory therapies that might enhance the therapeutic impact of dendritic cells, ultimately contributing to more personalized and effective treatment strategies for DKD.

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Clinical Trial: Effect of Autologous Dendritic Cell Administration on Improving Neuropathy Symptoms and Inflammatory Biomarkers in Diabetic Neuropathy

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1. Introduction

Diabetic neuropathy (DN) is one of the common complications of Type 2 diabetes mellitus (T2DM) that has a high prevalence. In China, the prevalence of DN in T2DM patients has reached 67.6%, and is especially found in the elderly and in low-income and low-education groups [1]. In Indonesia, about 28% of T2DM patients have DN, with studies showing no significant association between duration of the diabetes and the incidence of neuropathy [2]. DN also accounts for 35% of microvascular complications, where risk factors such as advanced age play an essential role in their development [3,4]. This high prevalence of DN demonstrates the importance of early screening and managing risk factors to prevent further complications.

The Toronto Clinical Neuropathy Score (TCNS) is used as a clinical assessment instrument that assesses the severity of neuropathy, especially the symptoms and sensory deficits that are often the initial manifestations of DN [5]. The TCNS has been validated by various measures of neuropathy, such as sural nerve fiber density and electrophysiological parameters, showing significant correlations, which make it a reliable tool for diagnosing and staging diabetic neuropathy [6]. Electromyography (EMG) complements TCNS by providing objective data on nerve function and muscle activity, and it can detect nerve damage that is not clinically apparent [7]. The combination of TCNS and EMG improves the accuracy of diagnosis and is highly beneficial in managing diabetic neuropathy.

Current management for DN focuses on prevention via blood sugar control, lifestyle changes, and pain management. Based on the evidence, blood sugar control only reduces the relative risk of DN by 5–9% and must be accompanied by lifestyle changes for more effective prevention. Also, the few existing guidelines on DN pain management include it together with other neuropathic pain, leading to inconsistencies [8]. Therefore, there is a need to develop DN therapies that target its pathogenesis.

Diabetes causes nitric oxide (NO) deficiency; activates alternative metabolic pathways; results in the accumulation of glycation end products (AGEs), oxidative stress, and inflammation through inflammatory molecules; and increases the expression of pro-inflammatory cytokines.

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Chronic hyperglycemia exacerbates cytokine infiltration in vascular tissue, inhibiting neuronal repair [9]. Autologous dendritic cells (DCs) from the immune system serve as antigen-presenting and potential anti-inflammatory agents, with studies showing the benefits of autologous DCs in diseases such as arthritis and autoimmune disorders as well as their potential to relieve peripheral nerve inflammation [10,11,12,13]. This study aimed to determine the changes in TCNSs and the anti-inflammatory effects following autologous DC administration in DN patients.

2. Materials and Methods

2.1. Study Design

This study is a quasi-experimental clinical trial with a pre-test and post-test design that aims to evaluate changes in the TCNS in DN patients after autologous dendritic cell administration. The study was conducted at Gatot Soebroto Army Central Hospital (RSPAD GS) on a population of DN patients undergoing outpatient care at the Internal Medicine Polyclinic. The Health Research Ethics Committee of RSPAD GS approved the study under Ethical Approval No:102/VIII/KEPK/2024. Before the start of the study, all the subjects were given clear information about the purpose, procedures, and risks and benefits of this study. The subjects were allowed to ask questions, and after fully understanding, they signed a written informed consent form as a sign of their agreement to participate in this study. Research ethics were applied to protect the subjects' rights during the clinical trial process.

2.2. Study Subjects

The study subjects consisted of DN patients who met the inclusion criteria and who were outpatients at the polyclinic of RSPAD GS during April 2024. The sampling technique used was consecutive sampling, in which subjects who met the criteria were included in the study sequentially until the minimum quota of 28 subjects was met. With this number of subjects, significant changes in TCNSs, which are used as the main indicator to measure the level of neuropathy in subjects, are expected to be detected.

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Eligible participants had to (1) be adults over 18, (2) be willing to comply with all study procedures and to provide written informed consent, (3) be capable of adhering to the study's protocols, (4) be in good physical and mental health, (5) meet the diagnostic criteria for Type 2 diabetes mellitus according to the PERKENI 2021 guidelines, and (6) their electromyography (EMG) results should be consistent with diabetic neuropathy.

Exclusion criteria included (1) recent immunosuppressive treatment, (1) non-diabetic neuropathy, (3) other types of diabetes, (4) pregnancy, (5) immunodeficiency (HIV, HCV, HBV), (6) ongoing invasive cancer treatment, (7) thromboembolism history, or (8) physical/mental disabilities hindering participation. Those unwilling to sign consent were also excluded.

2.3. Participant Characteristics

The recruitment process began with 3103 patient visits to the RSPAD Internal Medicine Polyclinic in April 2024. Among these, 276 patients were from the Endocrine Metabolic Diabetes Clinic, and 390 were from the Nephrology Clinic. Following a screening based on the inclusion criteria, 156 patients qualified, while 510 did not meet the inclusion criteria. Of the 156 eligible patients, 30 agreed to participate in the study, while 126 declined. Out of the 30 consenting participants, 29 proceeded to participate in the study; however, 1 was excluded due to meeting the exclusion criteria. During the study, 1 participant was lost to follow-up, resulting in a total of 28 subjects who completed the study and who were analyzed.

In total, 28 subjects satisfied both the inclusion and exclusion criteria and completed the entire study protocol. The average age of the participants was 61 years, with a gender distribution of 9 men (32%) and 19 women (68%). Hypertension was the most common comorbidity, affecting 26 participants (93%). Most participants fell into the overweight category, with a body mass index indicative of this in 35 subjects (50.7%). Additionally, a majority of participants, 20 individuals (71%), were using insulin (Table 1).

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Table 1. Subjects' baseline characteristics.

Baseline Characteristics		
Number of subjects		28
Gender, n (%)	Men	9 (32)
	Women	19 (68)
Age, mean		61 ± 9.5
Comorbidities, n (%)	Hypertension	26 (93)
	Heart disease	11 (39)
	Stroke	1 (4)
	Osteoarthritis	8 (8)
BMI, n (%)	Underweight	2 (7)
	Normal weight	8 (29)
	Overweight	13 (46)
	Obese	5 (18)
Types of Anti-diabetics, n (%)	Sulfonylurea	9 (32)
	Biguanide	7 (25)
	α-Glucosidase inhibitor	5 (18)
	DPP4 inhibitors	3 (11)
	SGLT2 inhibitors	4 (14)
	Insulin	20 (71)

2.4. Study Procedure

Each recruited subject participated in a 5-week clinical trial. The study began with a screening phase to confirm eligibility for participation. Once deemed eligible, subjects underwent a baseline examination, which included blood collection for DC preparation, serum collection for Transforming Growth Factor-β (TGF-β) and Vascular Cell Adhesion Molecule-1 (VCAM-1) measurement, EMG examination to confirm the

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diagnosis of DN, and TCNS assessment. Additional initial laboratory tests were also conducted at this stage. Seven days after the blood collection, each subject received a subcutaneous DC injection in the deltoid region. Finally, four weeks following DC administration, a follow-up assessment was conducted, including serum collection, TCNS measurement, and repeat laboratory tests.

2.5. Clinical and Laboratory Assessments

The TCNS is a widely recognized tool for assessing the severity of DN. The TCNS consists of 13 assessment points organized into three subsections: (1) Symptom Score—a subjective, patient-reported subsection that evaluates symptoms such as numbness, tingling, and pain, with each scored on a scale from 0 to 1, reflecting the presence or absence of specific symptoms; (2) Reflex Score—an objective, examiner-assessed score focusing on ankle reflexes that is scored from 0 to 2, where 0 represents normal reflexes, 1 indicates decreased reflexes, and 2 signifies absent reflexes; and (3) Sensory Score—also examiner-assessed, this subsection evaluates the patient's sensory function, including responses to vibration, pinprick, and temperature sensations, with each scored from 0 to 1 based on the presence or absence of sensation in specific areas of the feet. The total TCNS ranges from 0 to 19, with higher scores indicating a greater severity of neuropathy, categorized into mild (1–8), moderate (9–12), and severe neuropathy (13–19).

In this study, a single trained physician conducted all the TCNS assessments to ensure consistency and to minimize inter-rater variability. To confirm the DN diagnosis and to objectively assess nerve function, EMG, a reliable diagnostic tool for detecting nerve damage and measuring nerve conduction, was also performed. Two senior neurologists conducted the EMG examinations, focusing on the sural nerve (sensory) and tibial nerve (motor). Both nerves were assessed through measurements of the motor nerve conduction velocity and amplitude.

For laboratory biomarker testing, blood samples were collected to measure TGF-β and VCAM-1 levels. Both biomarkers were quantified using sandwich enzyme-linked immunosorbent assay (ELISA) kits, which offer high sensitivity and specificity for detecting these proteins in serum.

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Additionally, fasting blood glucose levels for each participant were measured. Blood samples were collected after an overnight fast (at least 8 h) to ensure accurate fasting glucose levels.

2.6. Autologous DC Preparation

A sample of 40 milliliters of peripheral blood was drawn from each participant. From this sample, peripheral blood mononuclear cells (PBMCs) were isolated and then cultured with Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) and Interleukin-4 (IL-4) over five days to generate immature DCs. These were further incubated with the antigen for two additional days to induce maturation of these cells. The number of dendritic cells administered varied for each participant depending on the yield of DCs obtained from their blood sample. No adjustments were made to standardize the cell count across participants. Instead, the entire DC preparation derived from each 40 mL sample was administered, resulting in a dose range of approximately 0.5 to 8 million DCs, depending on the individual yield.

2.7. Clinical Evaluation and Laboratory Testing

After the injection, an evaluation of the TCNS was performed in week 4. In addition to the laboratory evaluation, the detection of inflammatory biomarkers, TGF-β, and VCAM-1 was performed at baseline and at 4 weeks after injection to observe changes in the inflammatory response. This study aims to evaluate the effect of autologous dendritic cell intervention on the diabetic neuropathy condition through various clinical and laboratory parameters.

2.8. Statistical Analysis

The statistical analysis for this study employed a range of tests to thoroughly assess the data from multiple perspectives. First, the Kolmogorov–Smirnov test was applied to determine the normality of the data distribution. For data that followed a normal distribution, a paired t-test was conducted to examine differences before and after the intervention. For non-normally distributed data, the Wilcoxon signed-rank test was utilized to compare values before and after the intervention. Additionally, Pearson correlation analysis was used to explore

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relationships between key variables, specifically focusing on changes in the TCNS and inflammatory biomarkers. All statistical analyses were performed using IBM SPSS Statistics (version 22), with Microsoft Excel employed for data visualization.

3. Results

3.1. Changes in the TCNS

This study measured the TCNS before and after the intervention. The mean TCNS before the intervention was 8.93, with a standard deviation of 2.73. After the intervention, there was a decrease in the mean TCNS to 7.5, with a standard deviation of 3.03. A normality test was conducted using the Shapiro–Wilk test before analyzing the difference between the before- and after-intervention scores. The p-value for the TCNS data before intervention in the normality test was 0.753, and the p-value for the after-intervention data was 0.775, indicating that both data sets were normally distributed.

A paired t-test was performed to test the hypothesis of a significant difference between the TCNSs before and after the intervention (Figure 1). The hypothesis test results showed a p-value of <0.001, indicating a statistically significant difference between the TCNSs before and after the intervention (Table 2). Additionally, the number of patients with mild neuropathy increased from 12 to 19, while the number of patients with severe neuropathy increased from 5 to 3 (Table 3; Figure 2).

Table 2. Changes in the TCNS, VCAM-1, and TGF-β.

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Variables	Mean (Std. Deviation)	*p* Value Hypothesis Test
TCNS Before	8.93 (2.73)	<0.001
TCNS After	7.5 (3.03)	
VCAM-1 Before	1389.75 ng/mL (368.12)	0.101
VCAM-1 After	1403.85 ng/mL (410)	
TGF-β Before	41.16 ng/mL (11.83)	0.835
TGF-β After	44,18 ng/mL (15.25)	

Variables were measured before and at 4 weeks after DC administration. The normality test was performed using the Shapiro–Wilk test, while the hypothesis test was performed using the paired *t*-test. Abbreviations: Toronto Clinical Neuropathy Score (TCNS); Transforming Growth Factor-β (TGF-β); Vascular Cell Adhesion Molecule-1 (VCAM-1).

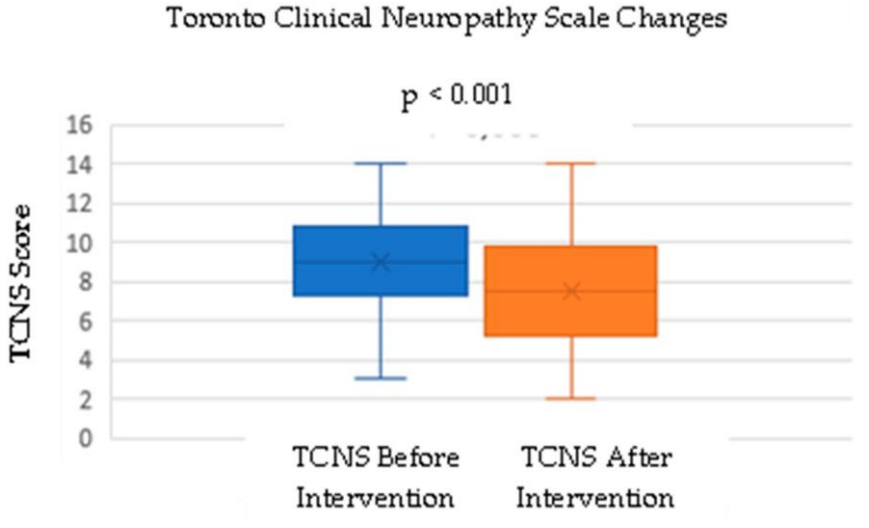

Figure 1. Change in the TCNS before and after DC administration.

Table 3. Diabetic neuropathy criteria shift.

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Diabetic Neuropathy Criteria (by the TCNS)	Before DC Administration	After DC Administration
Mild Neuropathy	12	19
Moderate Neuropathy	11	6
Severe Neuropathy	5	3

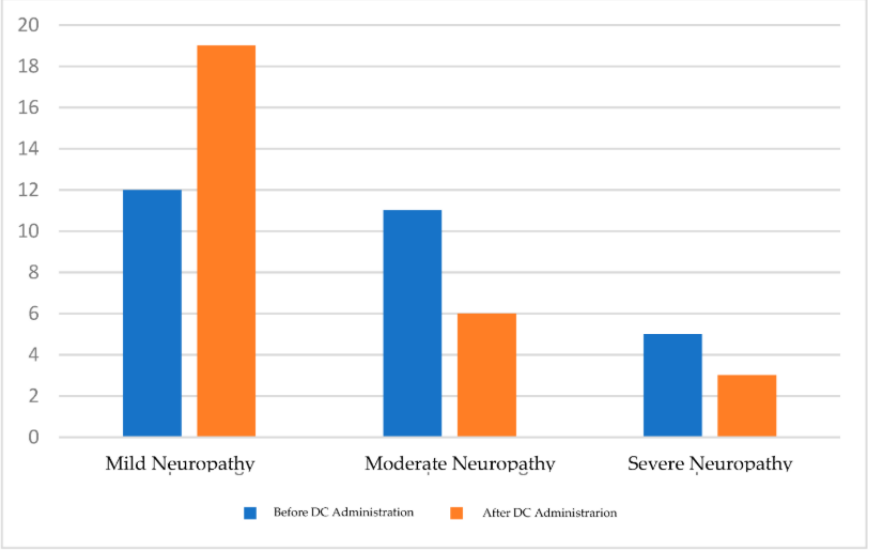

Figure 2. Diabetic neuropathy criteria shift.

3.2. Effect on Fasting Blood Glucose

The results of hypothesis testing using a paired t-test showed no significant difference between fasting blood glucose levels before and after the intervention. The average fasting blood glucose level before the intervention was 143.07 ± 54.2 mg/dL, while 4 weeks after intervention, it was 143.46 ± 47.1 mg/dL, with a p-value of 0.970 (Table 4). This indicates that the intervention did not significantly affect fasting blood glucose levels.

Table 4. Change in fasting blood glucose.

Variables	Mean ± SD	*p*-Value Hypothesis Test
Fasting Blood Glucose Before	143.07 ± 54.2	0.970
Fasting Blood Glucose After	143.46 ± 47.1	

Variables were measured before and at 4 weeks after DC administration. The normality test was performed using the Shapiro–Wilk test; the hypothesis test was performed using the paired *t*-test.

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3.3. Effect on VCAM-1 and TGF-β Levels

VCAM-1 levels increased slightly from a mean of 1389.75 ng/mL (SD = 368.12) before the intervention to 1403.85 ng/mL (SD = 410) after the intervention. A normality test using the Shapiro–Wilk method confirmed that both the before- and after-intervention data were normally distributed, with p-values of 0.419 and 0.169, respectively. A paired t-test was conducted to assess the significance of the change in VCAM-1 levels. The test yielded a p-value of 0.101 (Table 2; Figure 3), indicating that the increase in VCAM-1 levels was not statistically significant ($p > 0.05$). This suggests that the intervention did not have a significant impact on VCAM-1 levels, despite a small observed increase.

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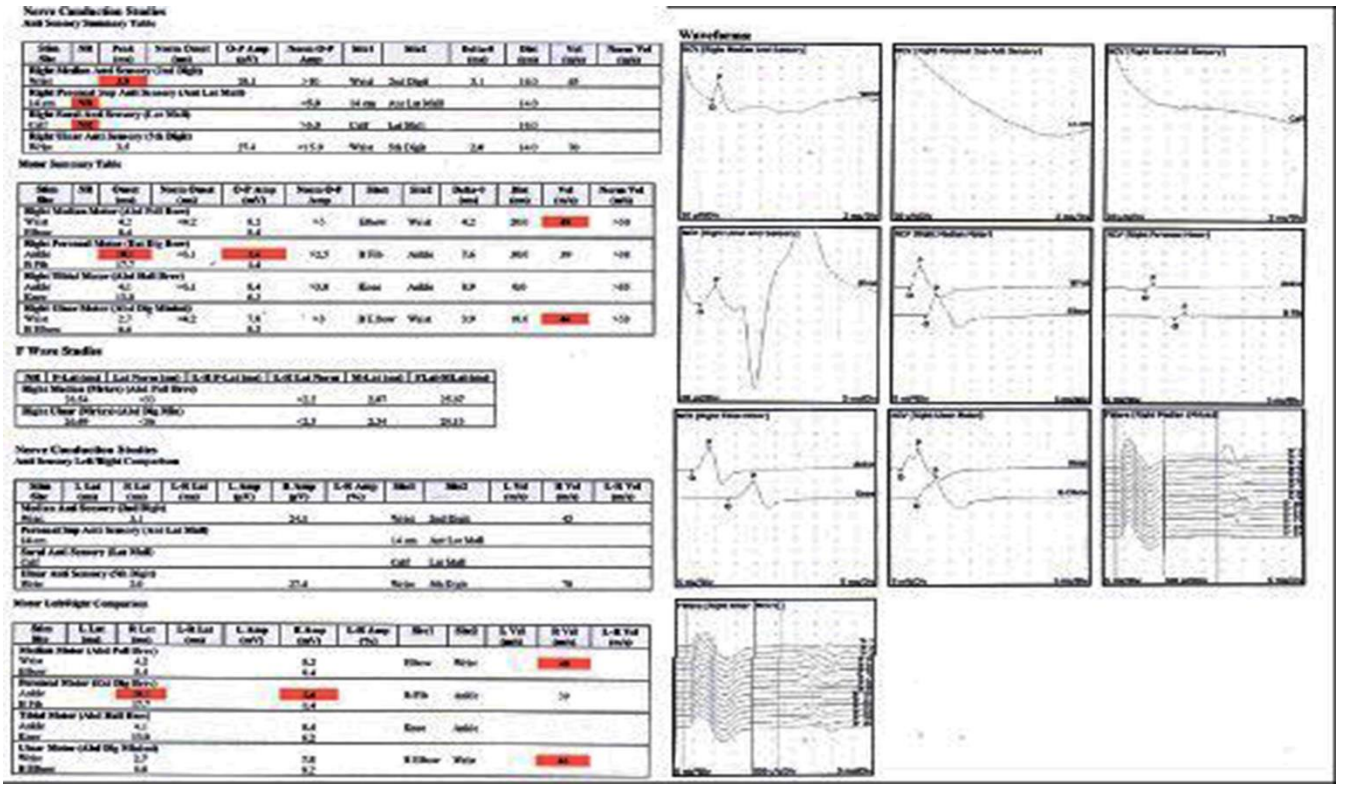

Figure 3. Example of an EMG study result. The red highlighted numbers means that the specific examination resulted in lower than normal value.

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For TGF-β levels, there was a slight increase from a mean of 41.16 ng/mL (SD = 11.83) before the intervention to 44.18 ng/mL (SD = 15.25) after the intervention. The normality test for TGF-β levels indicated that both the before- and after-intervention data were normally distributed, with p-values of 0.524 and 0.835, respectively. A paired t-test was also conducted to evaluate the difference in TGF-β levels before and after the intervention. The test resulted in a p-value of 0.835 (Table 2; Figure 4), showing that the increase in TGF-β levels was not statistically significant ($p > 0.05$). While there was a trend toward increased TGF-β levels after the intervention, this change was not statistically significant.

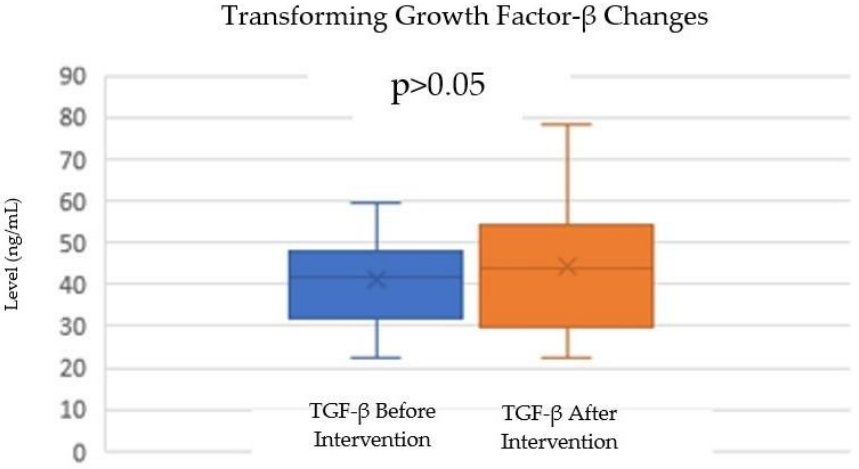

Figure 4. Change in TGF-β levels after autologous DC administration.

3.4. Correlation of the TCNS and TGF-β and VCAM-1 Levels

This study conducted correlation analyses to evaluate the relationships between VCAM-1 and TGF-β levels with the TCNS before and after the intervention. For VCAM-1 levels measured before the intervention, there was a weak positive correlation with TCNSs before ($r = 0.117$; $p = 0.277$) and after the intervention ($r = 0.125$; $p = 0.263$); however, these correlations were not statistically significant. A statistically significant moderate negative correlation was observed between VCAM-1 levels before the intervention and TGF-β levels before the intervention ($r = -0.338$; $p = 0.039$), indicating that higher VCAM-1 levels were associated with lower TGF-β levels prior to the intervention. VCAM-1 levels after

the intervention showed a weak, non-significant positive correlation with TCNSs both before (r = 0.264; p = 0.087) and after the intervention (r = 0.262; p = 0.089). Notably, VCAM-1 levels after the intervention were significantly negatively correlated with TGF-β levels both before (r = −0.521; p = 0.002) and after the intervention (r = −0.397; p = 0.018), suggesting an inverse relationship between these biomarkers (Table 5; Figure 5).

Table 5. Pearson's correlation test of variables.

	TCNS Before		TCNS After		TGF-β Before		TGF-β After	
	Correlation	*p*-Value	Correlation	*p*-Value	Correlation	*p*-Value	Correlation	*p*-Value
VCAM-1 before	0.117	0.277	0.125	0.263	−0.338	**0.039**	−0.197	0.158
VCAM-1 after	0.264	0.87	0.262	0.089	−0.521	**0.002**	−0.397	**0.018**
TGF-β before	−0.300	0.061	−0.326	**0.045**				
TGF-β after	−0.320	**0.048**	−0.353	**0.033**				

Variables were measured before and at 4 weeks after DC administration. Abbreviations: Toronto Clinical Neuropathy Score (TCNS); Transforming Growth Factor-β (TGF-β); Vascular Cell Adhesion Molecule-1 (VCAM-1). The bold is to highlight the significant *p*-values.

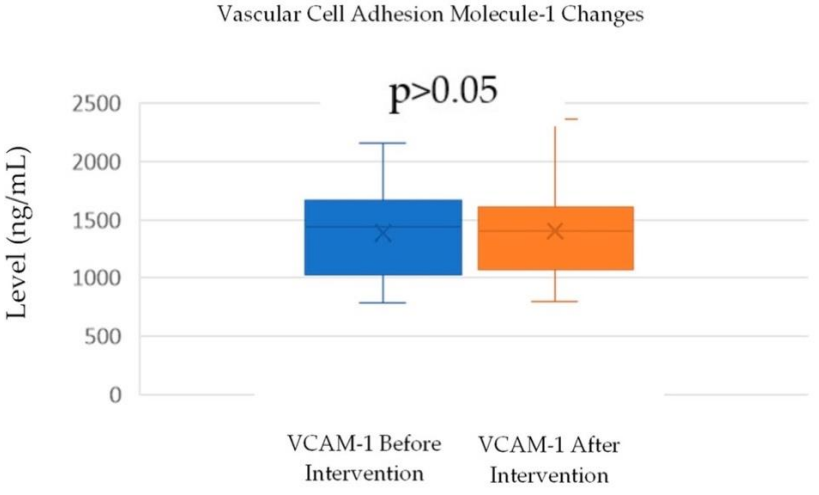

Figure 5. Change in VCAM-1 levels.

TGF-β levels before the intervention showed a moderate negative correlation with TCNSs before the intervention (r = −0.300; p = 0.061),

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although this correlation did not reach statistical significance. However, there was a statistically significant negative correlation between TGF-β levels before the intervention and TCNSs after the intervention ($r = -0.326$; $p = 0.045$), suggesting that higher baseline TGF-β levels were associated with lower neuropathy symptoms after the intervention. TGF-β levels after the intervention also showed statistically significant negative correlations with TCNSs both before ($r = -0.320$; $p = 0.048$) and after the intervention ($r = -0.353$; $p = 0.033$), indicating that higher TGF-β levels following the intervention were associated with lower TCNSs, reflecting reduced neuropathy symptoms (Table 5).

The subgroup analysis divided the subjects into two groups: one group with "no change" in the neuropathy criteria and one group with "improved" neuropathy criteria based on the TCNS. The "no change" in neuropathy group, as defined by the TCNS criteria, did not change from before and after the intervention, despite the TCNS reduction. In the "no-change" in neuropathy group, mean TGF-β levels increased by 0.821 ng/mL, but this was not statistically significant ($p > 0.05$). Meanwhile, the "improved" neuropathy group showed an average increase in TGF-β levels of 5.941 ng/mL ($p > 0.05$), indicating a trend toward more pronounced changes, even though this was not statistically significant. The difference between the two groups also shows no statistically significant result ($p > 0.05$) (Table 6; Figure 6).

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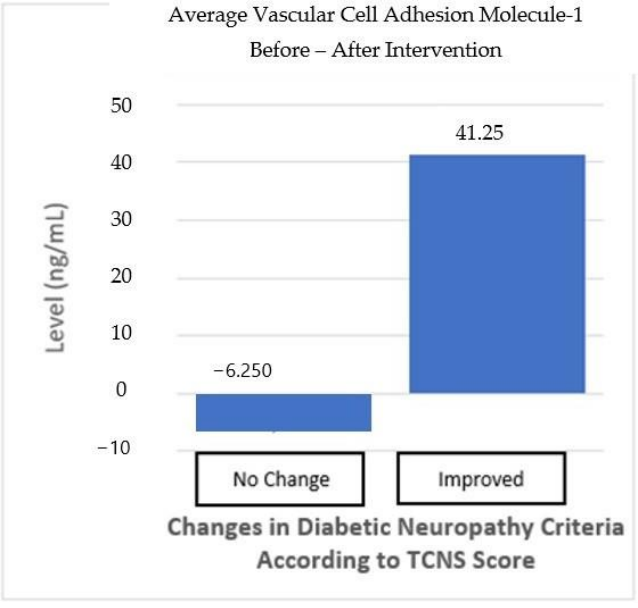

Figure 6. VCAM-1 average before and after the intervention based on improvements in the neuropathy criteria.

Table 6. Subgroup analysis of biomarker changes based on improvements in the neuropathy criteria.

Diabetic Neuropathy * Criteria	Mean TGF-β Change (SD)	*p*-Value [1]	*p*-Value [2]	Mean VCAM-1 Change (SD)	*p*-Value [1]	*p*-Value [2]
No Change *n* = 16	0.821 (8.1)	0.692	0.158	−6.25 (329.7)	0.941	0.733
Improved *n* = 12	5.941 (49.8)	0.076		41.25 (399)	0.727	

Variables were measured before and at 4 weeks after DC administration. Abbreviations: Transforming Growth Factor-β (TGF-β); Vascular Cell Adhesion Molecule-1 (VCAM-1). * Based on TCNS Score. [1] Paired *T*-test. [2] Independent Sample *T*-test between 2 groups.

In addition, changes in VCAM-1 levels were also analyzed for both groups. In the "no-change" in neuropathy group, VCAM-1 levels decreased by 6.25 ng/mL, but this decrease was not statistically significant ($p > 0.05$). In contrast, in the "improved" neuropathy group, there was an increase in VCAM-1 levels of 41.25 ng/mL, but this increase was also not significant ($p > 0.05$). The difference between the two groups also shows

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no statistically significant result ($p > 0.05$) (Table 6, Figure 7). Although the changes in TGF-β and VCAM-1 levels showed an improvement trend in the "improved" neuropathy group, these results were not statistically significant enough to be considered a definite effect of the intervention.

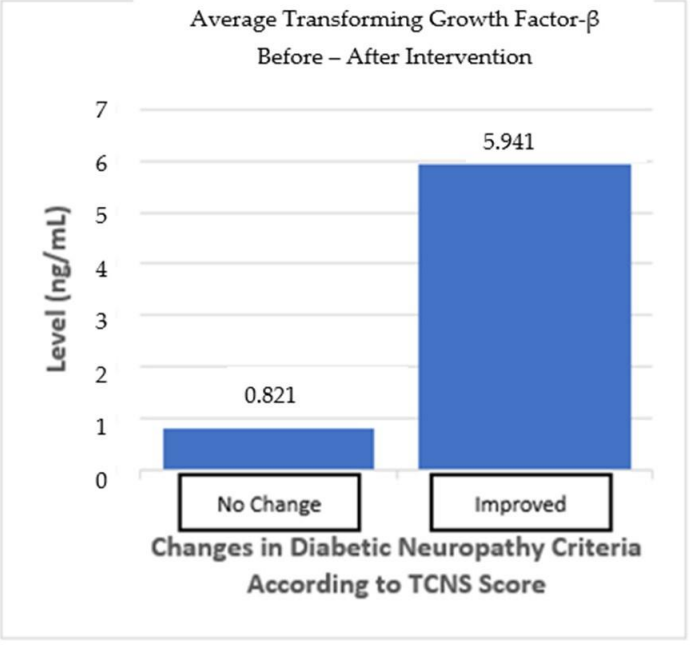

Figure 7. TGF-β average from before and after the intervention based on improvements in the neuropathy criteria.

4. Discussion

DCs serve as professional antigen-presenting cells that bridge innate and adaptive immunity. They can promote antigen-specific tolerance by inducing regulatory T-cells and suppressing pro-inflammatory responses [14]. This has been demonstrated in systemic lupus erythematosus (SLE), where autologous DC therapy led to significant clinical improvements and reductions in inflammatory markers [15]. The potential for using tolerogenic DCs in autoimmune diseases is highlighted by their capacity to reset immune dysregulation, offering long-term therapeutic effects without relying heavily on immunosuppressants [14,15].

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In infectious disease contexts, autologous DCs have been applied to elicit targeted immune responses, as seen in SARS-CoV-2 vaccine trials [16,17,18]. These studies showed that ex vivo-primed DCs can effectively activate T-cells, reduce systemic inflammation, and provide sustained immunity. This reflects their broader utility in managing chronic inflammatory states, including those involved in diabetic neuropathy.

Autologous dendritic cells represent a versatile tool in anti-inflammatory therapy. They offer a pathway for modulating immune activity, reducing inflammation, and potentially improving conditions like diabetic neuropathy through targeted, immune-regulating mechanisms.

This study showed a decrease in the mean TCNS after autologous dendritic cell administration. This decrease in the score reflects the improvement in DN symptoms experienced by patients. Research on diabetic mice showed similar results [19,20,21,22]. Natural anti-inflammatory agents have shown significant potential in managing diabetic neuropathy through various mechanisms. They reduce pro-inflammatory cytokines, oxidative stress, and gliosis while enhancing neurotrophic factors like brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), and nerve growth factor (NGF). These agents modulate pathways such as those of AMP-activated protein kinase (AMPK) and PPAR-γ, contributing to neuroprotection [19,20]. Additionally, they improve nerve function and alleviate inflammation by suppressing markers like TNF-α and IL-6, underscoring their promise in therapeutic applications for diabetic neuropathy [22,23].

Using additional anti-inflammatory agents also can reduce nerve hypersensitivity to mechanical and temperature stimuli better than by using insulin alone [24]. Studies have also shown that this anti-inflammatory effect modulates gut microbiota. The study showed that the increased production of short-chain fatty acids, such as butyrate, produced by certain gut bacteria, can strengthen the integrity of the gut barrier and reduce its permeability. This contributes to decreased levels of endotoxins such as lipopolysaccharide (LPS) in the bloodstream, significantly lowering the systemic inflammatory response. Reduced levels of pro-inflammatory cytokines such as TNF-α and IL-6 following gut microbiota

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intervention have also been shown to correlate with improved symptoms of diabetic neuropathy, including reduced pain and improved peripheral nerve function [25].

TGF-β plays a vital role in tissue repair and immunomodulation. Broader evidence shows that TGF-β plays an essential role in nerve repair and regeneration, especially after immunomodulating therapies such as the introduction of autologous dendritic cells. Echeverry et al. showed that TGF-β1 effectively reduced neuropathic pain in a mouse model by inhibiting neuroinflammation, preventing neuronal damage, and suppressing microglia and astrocyte cell activation, thereby reversing the conditions of mechanical allodynia and thermal hyperalgesia [26]. This study supports evidence of TGF-β's important neuroprotective and anti-inflammatory role in reducing neuropathic pain, suggesting its potential as a therapeutic target for treating various neuropathic conditions.

Clinically, these findings confirm that TGF-β may have an essential role in improving neuropathic conditions, especially after intervention. In the group of patients who experienced improvement in DN criteria, there was a more significant increase in TGF-β levels, although this was not statistically significant. As a growth factor involved in tissue repair processes and modulation of inflammatory responses, TGF-β may have a protective effect in peripheral neuropathy [27]. Although the correlations after intervention were insignificant, the results after intervention showed that TGF-β levels were associated with decreased TCNSs, indicating clinical improvement in patients. Similar results were reported by Ye et al., indicating that TGF-β has a strong correlation with the process of nerve regeneration. TGF-β not only helps facilitate debris clearance and the establishment of a supportive microenvironment but also directly increases the capacity of neurons to regrow [28]. As such, TGF-β is considered an essential factor in facilitating peripheral nerve recovery after injury [29].

In addition, there was a significant negative correlation between TGF-β and VCAM-1 levels, with moderate correlation strength. This is in line with the findings of Park et al., who stated that TGF-β and VCAM-1 have a relationship in which TGF-β acts as a regulator that suppresses VCAM-

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1 expression [30], which is usually induced by pro-inflammatory cytokines such as IL-1β and TNF-α in endothelial cells [31]. This study shows that TGF-β levels are inversely correlated with VCAM-1-expression, which is essential for recruiting immune cells to inflamed tissues. By suppressing the expression of VCAM-1, TGF-β1 plays a role in controlling the inflammatory response in endothelial cells, which could be very beneficial in inflammation control [31]. Furthermore, a study indicates that during peripheral nerve regeneration, TGF-β levels increase, peaking for approximately seven days. Therefore, the recommended evaluation after therapy should occur weekly for the first month [29].

Vascular Cell Adhesion Molecule-1 (VCAM-1) is critical in the pathophysiology of DN, serving as a mediator of inflammation and endothelial dysfunction. Hyperglycemia and oxidative stress in diabetes upregulate VCAM-1 expression, facilitating leukocyte adhesion to the endothelium and amplifying inflammation [32,33]. This contributes to nerve damage and the progression of DN. Elevated VCAM-1 levels correlate with the severity of diabetic complications, including neuropathy, nephropathy, and retinopathy [34]. The interaction of VCAM-1 with cytokines like TNF-α and IL-1 activates NF-κB signaling, further exacerbating inflammatory responses [35].

Those findings show some discrepancies with our study. This suggests that VCAM-1 levels may not always directly correlate with clinical improvements in diabetic neuropathy. The observed increase in VCAM-1 might be attributed to persistent hyperglycemia, leading to the formation of advanced glycation end products (AGEs), or, potentially, be due to insulin resistance [36,37]. Notably, our study showed no significant difference in fasting blood glucose (FBG) levels after treatment, which could indicate that hyperglycemia-induced oxidative stress or insulin resistance might be driving the increased VCAM-1 expression, independently of clinical improvements.

This study showed no significant changes in the subjects' fasting blood glucose levels before and after autologous dendritic cell administration. However, there was an improvement in DN symptoms, as indicated by a significant improvement in the TCNS. The improvement was also

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correlated with increased TGF- β levels as a marker of an enhanced anti-inflammatory response after the autologous dendritic cell administration. Although glycemic control is essential for managing diabetes, it is often insufficient to prevent or slow the progression of DN in T2DM. Strict glycemic control is also more effective in alleviating DN symptoms in Type 1 diabetes mellitus (T1DM) than in T2DM, which is still unclear [38]. DN can develop even in newly diagnosed patients, suggesting that factors other than hyperglycemia, such as oxidative stress and inflammation, play a role in nerve damage [39].

This study has several limitations. The small sample size and short follow-up period limit the generalizability and long-term applicability of the findings. The lack of a randomized control group prevents definitive conclusions about the intervention's efficacy, and conducting the study at a single center may reduce its representativeness. Additionally, the analysis was limited to a few biomarkers, leaving the broader mechanisms of action unexplored, and the study did not assess the effects of repeated or combined interventions.

5. Conclusions

This study shows that administering autologous dendritic cells significantly improves neuropathy symptoms by substantially decreasing the TCNS, reflecting improved DN symptoms. Although there was a trend toward increased VCAM-1 and TGF-β levels, these changes were not statistically significant. However, these findings indicate that autologous dendritic cells might exert anti-inflammatory effects and support nerve regeneration. The significant negative correlation between TGF-β and VCAM-1 corroborates the role of TGF-β in suppressing the expression of inflammatory molecules, reducing inflammation, and in promoting nerve repair.

In addition, research results show that glycemic control alone is not enough to prevent or improve diabetic neuropathy effectively. Other factors, such as inflammation and oxidative stress, play a role in the development of DN, even in patients with controlled glucose levels. Through their immune modulation and anti-inflammatory role, autologous

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dendritic cells show potential as adjunctive therapies for treating diabetic neuropathy. Thus, although further studies are needed to validate these findings, this study suggests that autologous dendritic-cell-based therapy may be a promising complementary approach for managing diabetic neuropathy through inflammation amelioration and nerve regeneration.

Future studies should include larger multicenter cohorts with longer follow-up periods and randomized controlled designs. Incorporating additional biomarkers and evaluating patient-reported outcomes will also enhance the robustness and clinical relevance of these findings.

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