

Impact of probiotic supplementation on serum klotho levels and inflammatory status in patients with diabetic kidney disease

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Abstract

Diabetic kidney disease (DKD) involves complex metabolic and inflammatory pathways, where gut dysbiosis via the gut-kidney axis plays a critical pathophysiological role. Probiotics may resolve renal decline and modulate aging biomarkers, but clinical evidence in DKD remains limited. This study evaluated the effects of Lactobacillus supplementation on serum alpha-Klotho levels, systemic inflammation, and renal biomarkers in DKD patients. In an open-label, parallel-group controlled clinical trial, conducted at the Nephrology Department of Eldakhla General Hospital, New Valley Governorate, Egypt on 80 adult DKD patients (stages G1A1 to G4A3) between August 2023 and January 2024 (recruitment: August 2023 – October 2023; intervention & follow-up: October 2023 – January 2024). Participants were assigned to an intervention group (n=40) receiving daily oral enteric-coated capsules containing Lactobacillus acidophilus and L. delbrueckii subsp. Bulgaricus, (5 billion CFU) /day for 12 weeks alongside standard care, a control group (n=40) receiving standard care alone. Primary outcomes were changes in serum alpha-Klotho (ELISA) and high-sensitivity C-reactive protein (hs-CRP). Secondary outcomes included serum creatinine, eGFR (CKD-EPI equation), and urinary albumin-to-creatinine ratio (UACR). Following intervention, serum alpha-Klotho levels significantly increased in the probiotic group compared to controls (adjusted post-treatment median: 114.15 ng/mL vs. 80.0 ng/mL; adjusted between-group $p=0.003$). Systemic inflammation markedly declined in the probiotic group relative to controls (hs-CRP post-treatment median: 2.1 mg/L vs. 6.9 mg/L; adjusted $p = 0.000$). Probiotic supplementation yielded significant relative decline in serum creatinine (0.97 ± 0.1 vs. 1.08 ± 0.23 mg/dl, $p=0.008$) and median UACR (24.4 vs. 69.05 mg/g $p = 0.000$). In addition, probiotic supplementation showed higher post treatment eGFR (81.35 ± 11.98 vs. 73.30 ± 18.07 ml/min/1.73 m² $p=0.021$). Reduction in HDL within-group was observed in both groups with no statistical significance between groups $p = 0.419$. No serious adverse events occurred. In conclusion, daily Lactobacillus supplementation for 12 weeks upregulates circulating serum alpha-Klotho, attenuates systemic hs-CRP, and improves clinical biomarkers of renal function in patients with diabetic kidney disease.

Keywords: Diabetic kidney disease; Probiotics; alpha-Klotho protein; Inflammation; Lactobacillus.

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Introduction

The global prevalence of diabetes is estimated to affect over 8% of the world population (>450 million people), with projections reaching 700 million by 2045. Approximately 40% of these patients develop diabetic kidney disease (DKD).¹ DKD is a major public health challenge characterized by elevated urine albumin excretion, reduced glomerular filtration rate (GFR), or both, involving complex hemodynamic, metabolic, and inflammatory pathways.² Despite advancements in clinical therapies, controlling DKD progression remains difficult, necessitating further study of its pathogenesis to improve patient prognosis.³

The Klotho protein functions as a Fibroblast growth factor 23 (FGF23) co-receptor and is primarily expressed on the cell surface membranes of renal tubules. Dysregulation of the FGF23-Klotho axis is strongly associated with chronic kidney disease (CKD) due to altered phosphate turnover, driving an accelerated aging phenotype.⁴ In the uremic milieu, lower klotho levels are exacerbated by protein-bound uremic toxins and elevated tumor necrosis factor (TNF), which impair klotho gene and protein expression. This relationship is mutually reciprocal; Klotho modulates anti-inflammatory responses via an NF- κ B-mediated pathway, whereas its own expression is downregulated by pro-inflammatory cytokines.⁴ Consequently, CKD displays an overlapping phenotype with klotho-deficient models, indicating that klotho function is tightly correlated with the pathogenic mechanisms underpinning renal decline.⁵

Inflammatory gut dysbiosis via the gut-kidney axis plays a key role in DKD pathogenesis. Probiotics like *Lactobacillus* spp. can attenuate this by reducing pro-inflammatory cytokines and upregulating tissue klotho expression.⁶ However, clinical studies evaluating these effects in diabetic nephropathy patients remain limited.

Therefore, the present study aimed to evaluate the effect of probiotic

supplementation on serum klotho protein levels, systemic inflammation (hs-CRP), and albuminuria in patients with diabetic kidney disease.

Materials and Methods

This open-label, parallel-group controlled clinical trial was conducted at the Nephrology Department of Eldakhla General Hospital, New Valley Governorate, Egypt, between August 2023 and January 2024 (recruitment: August 2023 – October 2023; intervention & follow-up: October 2023 – January 2024). The study was organized and operated strictly according to the guidelines of the International Council on Harmonization (ICH), the Islamic Organization for Medical Sciences (IOMS), and the United States Office for Human Research Protections (FWA 000017585). Furthermore, all research procedures were conducted in full accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Study Population Eighty adult patients (>18 years) with DKD were consecutively enrolled from outpatient clinics. DKD was defined according to KDIGO guidelines as persistent albuminuria (UACR >30 mg/g confirmed ≥ 2 consecutive morning urine samples collected over 3 months) and/or reduced eGFR (< 60 mL/min/1.73 m²) in patients with a history of Type 2 Diabetes Mellitus >5 years. Patients in stage G1A1 were included only if structural/histological evidence of diabetic nephropathy was clinically established. Non-diabetic renal diseases were excluded by medical history, serological screening, and renal ultrasonography. Patients were excluded if they had active infections, malignancy, autoimmune diseases, liver disease, advanced decompensated medical conditions (e.g., exacerbations of congestive heart failure or obstructive lung disease), were pregnant, had a history of renal transplantation or were on

dialysis, or had received antibiotic/antifungal treatment within 14 days prior to baseline.

Study Design and Randomization

Participants were allocated in a parallel manner to either the intervention group ($n = 40$) or the control group ($n=40$). Eligible participants were randomly allocated in a 1:1 ratio to either the probiotic intervention group or the control group (standard medical care alone) using a computer-generated randomization sequence.

Intervention Protocol and Treatment Compliance

The intervention group received a daily oral enteric-coated capsule of Lacteal Forte (Chemical Industries Development [CID], Giza, Egypt) containing *Lactobacillus acidophilus* (3×10^9 CFU) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (2×10^9 CFU), establishing a total daily dose of 5×10^9 CFU/day for a standardized 12-week intervention period alongside standard medical care. The control group received usual standard care without a placebo capsule due to local manufacturing constraints. Treatment adherence was evaluated via bi-weekly pill counts at follow-up visits; adherence $> 85\%$ of the prescribed doses was required for final analysis.

Laboratory Measurements Laboratory investigations were performed at baseline and repeated immediately after the 12-week treatment course. Venous blood samples were collected after a 12-hour fast. Assayed parameters included serum creatinine, serum urea, haemoglobin, platelets, lipid profile (total cholesterol, triglycerides, LDL, HDL), HbA1c, serum potassium, and high-sensitivity C-reactive protein (hs-CRP). The estimated glomerular filtration rate (eGFR) was calculated using the 2009 CKD-EPI creatinine equation. First morning void urine samples were collected to calculate the urinary albumin to creatinine ratio (UACR). Transient causes of elevated UACR (fever, urinary tract infection, strenuous exercise) were ruled out prior to testing.

Serum alpha Klotho Quantification Serum alpha -Klotho levels were measured using a commercially available quantitative Sandwich Enzyme-Linked Immunosorbent Assay (ELISA)

kit (Shanghai SunRed Biotechnology Co., Ltd., Shanghai, China; Catalog No. SRB-E10293). The assay specifically quantifies human soluble alpha-Klotho. Venous blood samples collected at baseline and at 12 weeks were centrifuged at 3,000 rpm for 10 minutes, and the separated serum aliquots were immediately frozen and stored at -80°C until batch analysis to ensure sample stability and avoid freeze-thaw cycles. Assays were performed according to the manufacturer's protocol, with a sensitivity threshold of $<0.1\text{ng/ml}$ and an operational detection range of 0.15 to 30 ng/ml. Samples exceeding the upper detection limit were diluted appropriately prior to measurement. Intra-assay and inter-assay coefficients of variation were $< 8\%$ and $< 10\%$, respectively.

Sample Size Calculation Sample size estimation was conducted using G*Power software (version 3.1.9.7). Based on preliminary data for changes in circulating Klotho levels following metabolic interventions, an effect size $d = 0.65$ was anticipated. Assuming a two-sided significance level $\alpha = 0.05$ and a power $1 - \beta$ of 80%, a minimum sample size of 38 participants per group was determined. To accommodate a potential 5% dropout rate, 80 participants (40 per group) were recruited.

Statistical Analysis

Data were analysed using IBM SPSS software version 28.0 (Armonk, NY: IBM Corp). The Shapiro-Wilk test and visual inspection of Q-Q plots were used to test data normality. Quantitative variables are expressed as mean $\pm$ standard deviation (SD) for parametric data, and as median and interquartile range (IQR; 25th–75th percentiles) for non-parametric data. Categorical variables are presented as absolute numbers and percentages (n , %). To evaluate between-group differences at week 12 while adjusting for baseline values and potential confounding factors, Analysis of Covariance (ANCOVA) was performed. In each ANCOVA model, the post-treatment parameter was set as the dependent variable, adjusted for its baseline value, baseline hypertension status, and baseline ACE inhibitor/ARB therapy. Highly skewed parameters (UACR, hs-CRP, serum alpha- Klotho) were log-transformed prior to

linear regression/ANCOVA modelling. Within-group changes from baseline to follow-up were evaluated using paired Student's t-tests or Wilcoxon signed-rank tests as appropriate. Categorical parameters were compared using Pearson's chi-square test or Fisher's exact test. Bivariate correlations were evaluated using Pearson (r) or Spearman (rho) coefficients. All p-values were two-tailed, and statistical significance was set at $p < 0.05$.

Results

Baseline Demographics and Clinical Characteristics

Among the study population (40 in the intervention group and 40 in the control group). There were no statistically significant differences between the two groups regarding age (60.22 ± 8.93 vs. 61.80 ± 11.13 years, $p=0.487$), gender distribution, body mass index, duration of diabetes, or baseline SGLT2 inhibitor use. However, baseline hypertension prevalence was significantly higher in the cases compared to controls (60.0% vs. 37.5% , $p=0.044$), the baseline demographic and clinical characteristics of the studied groups are shown in table 1.

Table 1. Baseline demographic and clinical characteristics of the studied groups.

Parameter	Cases (n = 40)	Control (n = 40)	p-value
Gender (Male/Female)	25 / 15	18 / 22	NS
Age (Years) [Mean $\pm$ SD]	60.22 ± 8.93	61.8 ± 11.13	NS
BMI (kg/m^2) [Mean $\pm$ SD]	29.06 ± 4.49	30.79 ± 4.75	NS
Duration of DM (Years) [Median (IQR)]	14 (12–20)	15 (10–15)	NS
Comorbidities n (%)			
Hypertension (HTN)	24 (60%)	15 (37.5%)	0.044
Ischemic Heart Disease (IHD)	6 (15%)	4 (10%)	NS
Dyslipidaemia	3 (7.5%)	0 (0%)	NS
Drug History n (%)			
SGLT2 inhibitors	12 (30%)	12 (30%)	-
ACEI or ARBs	24 (60%)	16 (40%)	-

$p > 0.05$ is not significant (NS).

Impact of probiotics on Kidney Function Parameters and Inflammatory Markers

Following 12 weeks of probiotic supplementation, intervention group exhibited significant improvement in renal functions biomarkers. Serum creatinine significantly decreased from 1.16 ± 0.53 to 0.97 ± 0.10 mg/dL ($p=0.026$), serum urea decreased from 37.73 ± 17.32 to 31.07 ± 8.48 mg/dL ($p=0.030$). Median UACR decreased from 35.4 to 24.4 mg/g ($p=0.039$), (Figure 1), while eGFR increased from 67.77 ± 20.90 to 81.35 ± 11.98 mL/min/ 1.73 m^2 ($p=0.000$). Adjusted between-group ANCOVA post-intervention demonstrated that the

intervention group achieved significantly lower serum creatinine ($p=0.008$), lower urea ($p=0.001$), lower UACR ($p=0.000$), and higher eGFR ($p=0.021$) compared to controls (Table 2, Figure 2). Systemic inflammation markedly decreased in the intervention group, with hs-CRP median declining to 2.1 mg/L post-treatment ($p=0.000$) (Figure 3). Compared to controls median serum alpha-Klotho levels increased significantly in the intervention group from 69.60 to 114.15 ng/mL ($p=0.001$), remaining significantly higher than control levels at after treatment ($p=0.003$) (Table 3, Figure 4).

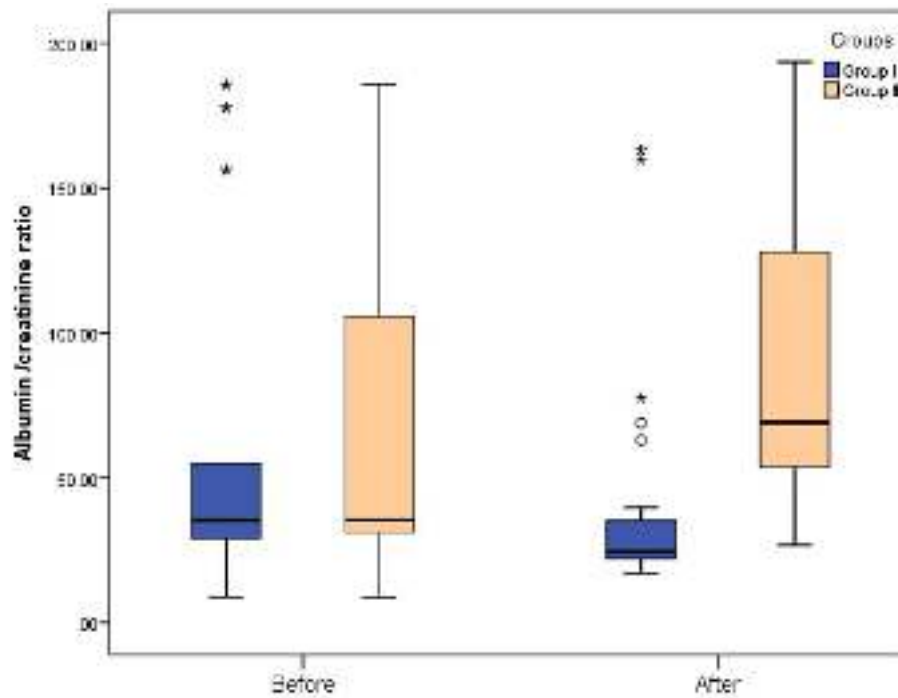


Figure 1. Impact on UACR. The figure shows the baseline ("Before") and 12-week post-treatment ("After") levels of Urinary Albumin-to-Creatinine Ratio (UACR in mg/g) in the probiotic intervention group (Group I, blue) compared to the control group (Group II, orange). The intervention group demonstrated a statistically significant reduction in median UACR following 12 weeks of daily *Lactobacillus* supplementation compared to controls.

Table 2. Changes in kidney function parameters and Inflammatory Markers before and after treatment.

Parameter	Cases (Before)	Cases (After)	Within <i>p</i> -value	Control (Before)	Control (After)	Within <i>p</i> -value	Between Groups <i>p</i> -value (After)
S. Creatinine (mg/dL)	1.16 ± 0.53	0.97 ± 0.10	0.026	1.13 ± 0.44	1.08 ± 0.23	NS	0.008
Urea (mg/dL)	37.73 ± 17.32	31.07 ± 8.48	0.030	37.09 ± 14.97	36.61 ± 5.48	NS	0.001
UACR (mg/g) [Median (IQR)]	35.4 (29-55)	24.4 (22.1-35.2)	0.039	35.6 (31.1-105.6)	69.05 (53.7-127.8)	NS	0.000
eGFR (mL/min/1.73m ²)	67.77 ± 20.9	81.35 ± 11.98	0.000	68.25 ± 19.56	73.3 ± 18.07	NS	0.021
S. Potassium (mmol/L)	4.44 ± 0.40	3.97 ± 0.45	0.000	4.40 ± 0.42	4.30 ± 0.21	NS	0.000
hs-CRP (mg/L) [Median (IQR)]	2.1 (1.25-11.4)	2.1 (1.2-5.2)	0.026	2.0 (1.2-12.1)	6.9 (5.6-8.0)	NS	0.000

p > 0.05 is not significant (NS).

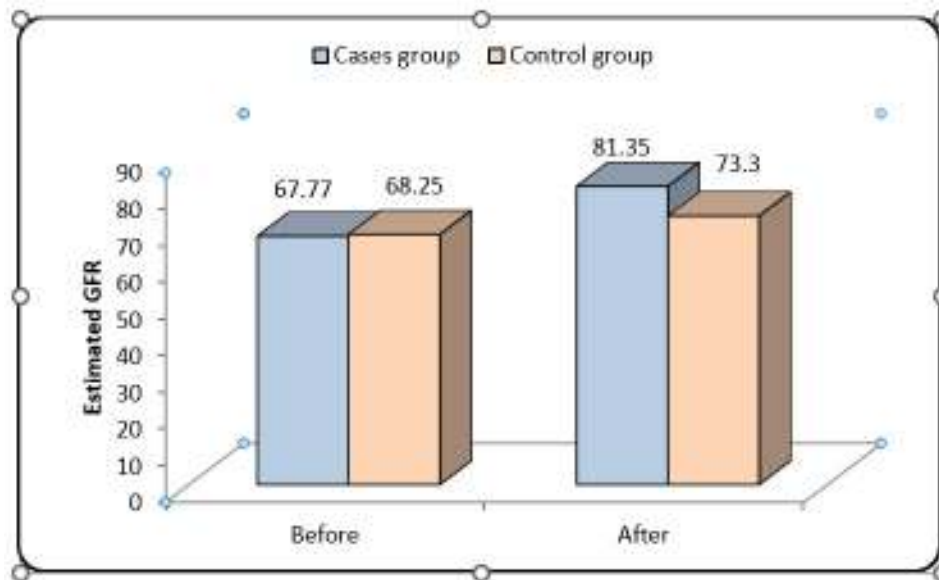


Figure 2. Impact on eGFR. The figure shows the impact of Probiotic Supplementation on Estimated Glomerular Filtration Rate (eGFR), comparing mean estimated Glomerular Filtration Rate (eGFR in mL/min/1.73m², calculated via the 2009 CKD-EPI equation) before and after 12 weeks of treatment in the cases group (blue) and control group (orange). Post-intervention eGFR significantly improved in the probiotic group (81.35±11.98 mL/min/1.73m²) compared to the control group 73.30±18.07 mL/min/1.73m²).

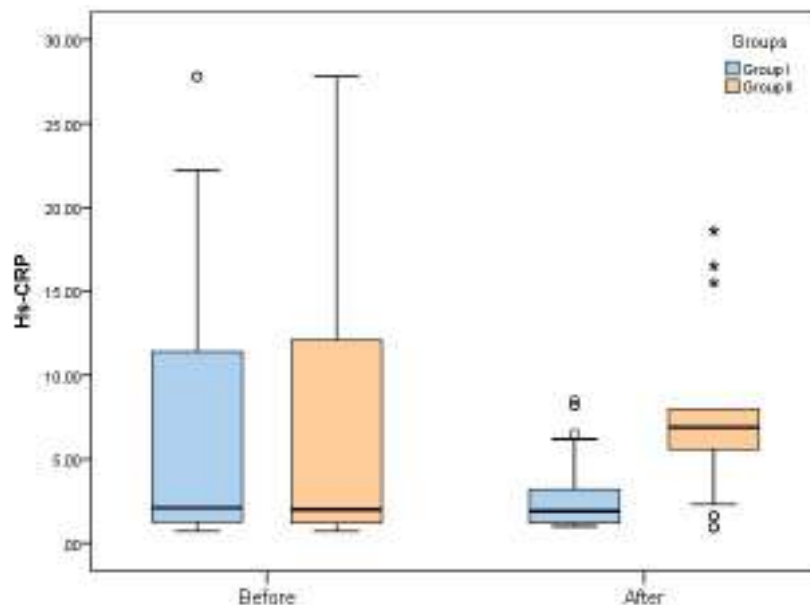


Figure 3. Impact on Systemic Inflammation. The above figure shows the impact of Probiotic Supplementation on Systemic Inflammation (hs-CRP), illustrating the changes in high-sensitivity C-reactive protein (hs-CRP in mg/L) baseline ("Before") and follow-up ("After") levels between Group I (probiotic intervention, blue) and Group II (control, orange). A marked reduction in systemic inflammation was observed in the probiotic arm post-treatment (median 2.1 mg/L vs. 6.9 mg/L in controls).

Table 3. Changes in Serum Klotho Protein (ng/mL) [Median (IQR)].

Group	Before Treatment	After Treatment	Within Group <i>p</i> -value
Cases (n = 40)	69.6 (45.2-120)	114.15 (88.3-474.3)	0.001
Control (n = 40)	70.5 (53.3-120)	80.0 (68.8-91.7)	NS
Between Groups <i>p</i> -value	NS	0.003	-

p > 0.05 is not significant (NS).

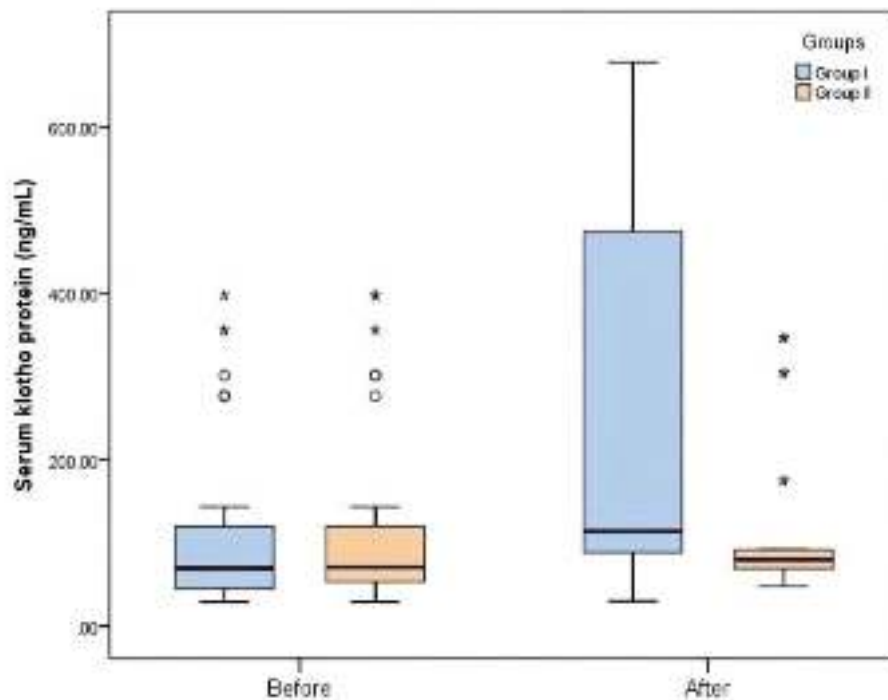


Figure 4. Impact on Serum Klotho. The figure shows the impact of Probiotic Supplementation on Serum Alpha-Klotho Protein Levels, demonstrating circulating serum alpha-Klotho protein concentration before and after the 12-week intervention period across Group I (probiotic intervention, blue) and Group II (control, orange). Daily *Lactobacillus* administration led to a statistically significant upregulation of serum alpha-Klotho levels in the intervention group compared to controls.

Metabolic Parameters and Safety

Compared to controls haemoglobin levels increased significantly in the intervention group from 12.85 ± 1.56 to 14.10 ± 1.56 g/dL ($p=0.000$). For lipid profile, LDL cholesterol rose significantly in the control group ($p= 0.006$) unlike the intervention group. HDL cholesterol declined significantly in both groups (controls $p=0.002$ and intervention group $p= 0.000$).

HbA1c levels remained unchanged in both groups. Overall therapeutic compliance was 91.2% in the probiotic arm. No serious adverse events occurred. Mild transient gastrointestinal symptoms (flatulence/ bloating) were reported by 3 participants (7.5%) in the intervention group, which resolved spontaneously within the first 10 days without treatment discontinuation (Table 4).

Table 4. Changes in inflammatory, metabolic, and haematological markers.

Parameter	Cases (Before)	Cases (After)	Within <i>p</i> -value	Control (Before)	Control (After)	Within <i>p</i> -value	Between Groups <i>p</i> -value (After)
Hb (g/dL)	12.66 ± 1.75	12.46 ± 2.19	NS	12.85 ± 1.56	14.10 ± 1.56	0.001	0.000
Platelets (10 ³ /cmm)	295.25 ± 60.76	304.3 ± 72.37	NS	293.25 ± 64.39	279.4 ± 82.74	NS	NS
Cholesterol (mg/dL)	165.35 ± 34.6	163.03 ± 30.31	NS	162.66 ± 35.37	166.97 ± 39.18	NS	NS
LDL (mg/dL)	84.75 ± 26.52	94.29 ± 30.41	NS	85.42 ± 27.44	104.04 ± 27.46	0.006	NS
HDL (mg/dL)	44.67 ± 9.90	38.12 ± 6.82	0.002	44.89 ± 10.24	39.23 ± 5.28	0.000	NS
Triglycerides (mg/dL)	179.64 ± 106.5	153.05 ± 133.8	NS	161.76 ± 96.54	118.52 ± 62.93	0.027	NS
HbA1C (%)	9.31 ± 2.57	9.27 ± 2.87	NS	9.66 ± 2.52	8.86 ± 2.14	NS	NS

p > 0.05 is not significant (NS).

Baseline Correlation Analyses

At baseline, serum alpha-Klotho displayed a significant negative correlation with eGFR ($r = -0.294$, $p = 0.008$) and haemoglobin ($r = -0.296$, $p = 0.008$), and a significant positive correlation

with triglycerides ($r = 0.438$, $p = 0.000$), HbA1c ($r = 0.315$, $p = 0.004$), and platelets ($r = 0.304$, $p = 0.006$) across the study (Table 5). Following probiotic administration, these correlations were resolved.

Table 5. Correlation of Baseline Serum Klotho Protein with Clinical Parameters (Total Sample, n=80).

Parameter	<i>r</i> (Pearson Coefficient)	<i>p</i> -value
Age (Years)	-0.209	NS
Hemoglobin (Hb)	-0.296	0.008
Platelets (PLT)	0.304	0.006
Estimated GFR	-0.294	0.008
Triglycerides (TG)	0.438	0.000
HbA1C	0.315	0.004

p > 0.05 is not significant (NS).

Discussion

The present study demonstrates that treatment with Lactobacillus-based probiotic supplementation significantly attenuated systemic inflammation (hs-CRP) and upregulated circulating serum alpha-Klotho protein levels in diabetic kidney disease patients (DKD). Our findings revealed significant

improvements in serum creatinine, urea, UACR, and eGFR following probiotic therapy. These observed reno-protective outcomes strongly align with findings by Dai et al.⁷ and Wang et al.,⁸ who reported that probiotic supplementation attenuates uremic toxin burden with significant decline in serum creatinine, urea, and urinary protein

concentrations. Similarly, Firouzi et al.⁹ confirmed improvements in renal biomarkers after 12 weeks of probiotic administration. However, in interpreting these findings, caution is required as decreased serum creatinine may stem from diminished intestinal generation of gut derived toxic precursors or altered metabolic processing rather than nephron regeneration. This distinction clarifies why meta-analyses by Jia et al.¹⁰ and Thongprayoon et al.¹¹ found no significant renal improvements. This discrepancy as well may be attributed to variations in probiotic strains, daily dosages, or intervention durations across different trials. Systemic chronic inflammation is a fundamental driver of renal parenchymal injury in DKD. We observed a significant decrease in hs-CRP levels post-treatment, consistent with Dai et al.,⁷ who demonstrated that probiotics attenuate inflammation and oxidative stress. However, this contrasts with reports by Jia et al.¹⁰ and Viramontes-Hörner et al.,¹² where no significant changes in CRP were detected. A central and novel finding of our study is the highly significant upregulation of serum alpha-klotho protein following probiotic consumption, alpha-Klotho is an essential anti-aging protein predominantly expressed in renal tubular epithelial cells, whose decline exacerbates renal oxidative stress, tissue fibrosis, and vascular calcification. This supports experimental models by Kaushal & Kansal,¹³ which showed that probiotic administration prevented age-related klotho decline and increased its expression in renal tissues. Our clinical trial extends these findings to human DKD, suggesting that targeting gut dysbiosis can restore circulating alpha-Klotho levels. Furthermore, Darwish et al.¹⁴ suggested that multi-strain probiotics might yield more pronounced klotho upregulation than single strains. Regarding metabolic parameters, our study found no significant between-group differences in HbA1c, this lack of glycaemic improvement contrasts with Wang et al.⁸ and Dai et al.,⁷ who reported enhanced glycaemic control. Regarding haemoglobin, it rose significantly in the intervention group. Regarding lipid metabolism, a significant within-group decrease in HDL cholesterol was noted in both the probiotic and control arms, with no

significant post-treatment between-group difference. This suggests that the HDL change was a secular trend or secondary effect unlinked to the probiotic intervention itself. Nevertheless, lipid kinetics warrant ongoing evaluation in future probiotic trials. Baseline correlation analyses provided additional pathophysiological insights. We observed a significant negative correlation between klotho and eGFR, which aligns with Kim et al.,¹⁵ while the lack of correlation with urea and creatinine contradicts findings by Bob et al.¹⁶ and Inci et al.¹⁷ Moreover, the observed negative correlation with hemoglobin and positive correlation with platelets may reflect klotho's physiological inhibitory effect on erythropoiesis and iron regulation, as suggested by Madathil et al.¹⁸ and An et al.¹⁹ Additionally, baseline alpha-Klotho correlated positively with HbA1c levels, consistent with population observations by Ciardullo et al.,²⁰ who demonstrated in a population-based study that serum levels of soluble Klotho are positively associated with HbA1c percentages. Similarly, Zubkiewicz et al.²¹ reported a significantly altered level of alpha-Klotho that correlated directly with HbA1c levels, but contrasting with Zhang et al.²² who demonstrated that α -Klotho levels were significantly downregulated in patients with type 2 diabetes mellitus and were negatively correlated with HbA1c. Furthermore, our data demonstrated no statistically significant correlation between serum klotho and the duration of diabetes mellitus, in contrast with the findings of Gong et al.²³ who concluded that circulating Klotho levels are significantly and inversely correlated to both HbA1c and diabetes duration, an association that remained robust even after statistically adjusting for age. These conflicting reports underscore the complex metabolic regulation of Klotho protein in diabetic patients and highlight the need for further clinical investigation.

Limitations of the Study

1. Lack of Placebo Control: The control arm received standard care without a matching placebo capsule, which introduces potential open-label performance and expectation bias.

2. **Single-Center Sample Size:** Although appropriately powered according to our sample size calculations ($n = 80$), the single center setting in Eldakhla General Hospital may limit generalizability to broader ethnic or geographical demographics.

3. **Follow-up Duration:** A 12-week intervention evaluates short-term biomarker responses; long-term studies (6–12 months) are required to determine whether Klotho upregulation translates into hard clinical endpoints such as delayed progression to end-stage kidney disease (ESKD).

Recommendations

Based on the clinically significant outcomes of this study, the following recommendations are proposed for future research and clinical practice:

1. **Large-Scale Multi-Center Trials:** Future randomized controlled trials with larger cohorts and extended follow-up durations (e.g., 6 to 12 months) are strongly recommended to evaluate the long-term safety, efficacy, and durability of probiotic interventions in DKD.
2. **Biomarker Screening:** Clinical monitoring of serum Klotho protein and hs-CRP should be explored as potential routine biomarkers to assess the therapeutic response and rate of progression in patients utilizing gut-kidney axis modulators.

Conclusion

These findings suggest that 12-week *Lactobacillus* supplementation may improve circulating α -Klotho, systemic inflammation, and selected renal biomarkers in patients with DKD. Larger placebo-controlled studies with longer follow-up are required to confirm these findings and determine whether these changes translate into clinically meaningful renal outcomes.

Author Contributions

All authors contributed equally to the study conception, clinical design, patient recruitment, data collection, statistical analysis, manuscript drafting, and critical revision for intellectual content. All authors have read and approved the final version of this manuscript for submission.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Ethical approval

The study protocol was rigorously reviewed and officially approved by the Research Ethics Committee (REC) at the Faculty of Medicine, Ain Shams University, Egypt (Approval Reference Number: FMASU MD 45 / 2022).

Informed consent

Written informed consent was obtained from all individual participants included in the study prior to their enrolment and any clinical intervention.

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