

# ASSOCIATION OF CORRELATION BETWEEN TNF- $\alpha$ GENE EXPRESSION WITH IL-18, SYNDECAN-1, NT-PROBNP AND HSCRP LEVELS IN PATIENTS WITH END-STAGE RENAL DISEASE UNDERGOING HEMODIALYSIS

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## ABSTRACT

**Background:** End-stage renal disease (ESRD) is accompanied by uremia, chronic inflammation, endothelial injury and cardiovascular stress. Although hemodialysis effectively removes small water-soluble solutes, its immediate influence on inflammatory and endothelial biomarkers, and on the underlying transcriptional inflammatory program, is less predictable. This study evaluated serum interleukin-18 (IL-18), syndecan-1 (SDC-1), N-terminal pro-B-type natriuretic peptide (NT-proBNP) and high-sensitivity C-reactive protein (hsCRP), together with urea, creatinine and peripheral blood tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) gene expression, before and after hemodialysis, and compared both dialysis states with healthy controls. **Methods:** Thirty-four clinically stable adults receiving maintenance hemodialysis and 22 apparently healthy controls were studied. Blood was collected immediately before and after one dialysis session. Protein biomarkers were measured by sandwich ELISA and renal parameters by enzymatic colorimetric assays. TNF- $\alpha$  mRNA expression was quantified by real-time quantitative PCR (RT-qPCR) using GAPDH as the reference gene and an efficiency-corrected relative-quantification model, expressed as fold-change relative to the mean control value. Mann-Whitney U, Wilcoxon signed-rank, Spearman correlation and receiver-operating-characteristic (ROC) analyses were used. **Results:** Pre-HD patients had significantly higher IL-18, SDC-1, NT-proBNP, hsCRP, urea and creatinine than controls (all  $P \leq 0.001$ ). After dialysis, all four protein biomarkers remained significantly higher than controls. Urea and creatinine fell markedly after treatment (both  $P < 0.001$ ), whereas IL-18, SDC-1 and hsCRP did not change significantly; NT-proBNP increased significantly ( $P = 0.039$ ). TNF- $\alpha$  gene expression was approximately two-fold higher in Pre-HD (mean  $2.03 \pm 0.62$ -fold; median 1.95, IQR 1.61–2.34) and Post-HD patients (mean  $2.07 \pm 0.60$ -fold; median 2.09, IQR 1.58–2.47) than in controls (mean  $1.00 \pm 0.30$ -fold) (both  $P < 0.001$ ), with excellent discrimination from controls (AUC 0.96 and 0.95, respectively), but showed no significant paired change across the single dialysis session ( $P = 0.723$ ). Strong positive correlations were observed among several inflammatory, endothelial and cardiac markers. ROC analysis showed excellent discrimination for hsCRP and NT-proBNP. **Conclusion:** A single high-flux hemodialysis session produced effective small-solute clearance but did not normalize the protein or transcriptional inflammatory profile of ESRD. TNF- $\alpha$  mRNA was markedly upregulated in ESRD and remained elevated after treatment, mirroring the pattern seen for hsCRP, IL-18 and SDC-1. The findings support an integrated inflammatory endothelial, cardiovascular, and molecular phenotype in ESRD and suggest that multimarker assessment, extending from the circulating protein level to the gene-expression level, may provide more information than any single biomarker.

**KEYWORDS:** end-stage renal disease; hemodialysis; TNF- $\alpha$  gene expression; IL-18; syndecan-1; NT-proBNP; hsCRP; inflammation; endothelial glycocalyx

## 1. INTRODUCTION

Chronic kidney disease (CKD) is a major global health problem and progresses in a proportion of affected individuals to kidney failure requiring kidney replacement therapy. The KDIGO 2024 guideline emphasizes risk assessment according to cause, glomerular filtration rate and albuminuria, reflecting the multidimensional nature of CKD rather than reliance on a single laboratory value (Kidney Disease: Improving Global Outcomes [KDIGO] CKD Work Group, 2024). In advanced kidney failure, loss of excretory and regulatory capacity results in accumulation of nitrogenous solutes, disordered fluid balance, altered mineral metabolism and profound cardiovascular risk.

Maintenance hemodialysis is a life-sustaining treatment for ESRD. It is a very efficient method of correcting many derangements of the internal milieu, primarily through its ability to eliminate diffusible solutes and excess fluid. But this efficiency is met with drastic physiological change. Ultrafiltration decreases plasma volume on the circulatory side and changes in vascular filling can lead to hemodynamic stress as well as compromised perfusion of tissues. Kooman et al. As noted (2018), hemodialysis is a "type of extreme physiology" because patients repeatedly have to endure significant cardiovascular, thermal and respiratory challenges.

Chronic kidney disease (CKD) and long-term dialysis are accompanied by a prominent inflammatory state. Chronic inflammatory activation may be impacted by uremic retention, oxidative stress, comorbid disease, infection and intestinal dysbiosis and exposure to the extracorporeal circuit. Several studies have indicated that elevated inflammatory biomarkers in patients on dialysis relate to cardiovascular events, mortality and protein-energy wasting (Nowak & Chonchol, 2018). Reconciliation: High-sensitivity CRP and high-sensitivity troponin are multi-Xcharacteristics of long-term follow-up in maintenance hemodialysis patients (Loredana Antunović et al., 2017) In this population high-sensitivity CRP therefore serves as an easily assessed, clinically validated measure of systemic inflammatory activity.

IL-18 is an IL-1 superfamily pro-inflammatory cytokine. Its involvement in innate immune signaling has been reported to induce the production of interferon- $\gamma$  and other downstream inflammatory responses. The study discussed contributes evidence for the involvement of IL-18 signalling in both renal inflammation and also fibrotic processes as utilised by reviews on inflammatory kidney disease (Hirooka & Nozaki, 2021). Clinical studies have connectively linked IL-18 with poor outcomes in ESRD, further enhancing its potential utility as a marker of the inflammatory and oxidative state regarding the dialysis environment (Liu et al., 2022).

Another key pro-inflammatory cytokine involved in the pathogenesis and progression of CKD is tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ). Compared with healthy individuals, circulatory levels of TNF receptors in CKD and ESRD subjects have been demonstrated to correlate with disease progression, renal dysfunction severity, organ fibrosis and inflammation (Lousa et al., 2022), therefore are also proposed biomarkers for understanding the risk of mortality as well as cardiovascular risk factors due to advances made in our knowledge on the pathophysiological role played by TNF- $\alpha$  acting through TNF receptors 1 and 2. Inhibition of TNF- $\alpha$  signaling in animal models of kidney injury is a good match to ongoing inflammatory gene expression and renal fibrosis, supporting the notion that this cytokine plays a mechanistic role rather than being merely epiphenomenal (Taguchi et al., 2021). In some populations, genetic evidence can also implicate TNF- $\alpha$  promoter polymorphisms with susceptibility to ESRD, consistent with the notion that transcriptional regulation as well as downstream signaling of this cytokine contributes to disease risk (Anumas et al., 2025). Because circulating TNF- $\alpha$  protein is difficult to measure reliably in uremia, in part owing to impaired renal clearance of the cytokine and its soluble receptors, quantification of TNF- $\alpha$  messenger RNA (mRNA) in peripheral blood by real-time quantitative PCR (RT-qPCR) provides a complementary, clearance-independent readout of the transcriptional inflammatory program that is increasingly used alongside protein-based inflammatory markers in hemodialysis research (Okuyan et al., 2023).

The endothelial glycocalyx is another important target of CKD and hemodialysis-associated injury. This carbohydrate-rich layer contributes to vascular permeability, mechanotransduction, leukocyte interactions and vascular homeostasis. Syndecan-1 is an important heparan-sulfate proteoglycan of the glycocalyx, and its appearance in the circulation is commonly interpreted as evidence of glycocalyx degradation. Liew et al. (2021) demonstrated that circulating syndecan-1 and hyaluronan were highest in dialysis patients, intermediate in non-dialysis CKD and kidney-transplant recipients, and lowest in healthy controls, and that these glycocalyx markers correlated with uremic toxin burden and endothelial dysfunction markers. Subsequent work showed that syndecan-1 can rise during hemodialysis itself: Koch et al. (2020) linked intradialytic sodium changes with syndecan-1 shedding, while Kusuzawa et al. (2021) reported a significant increase in serum syndecan-1 after hemodialysis in a large cohort.

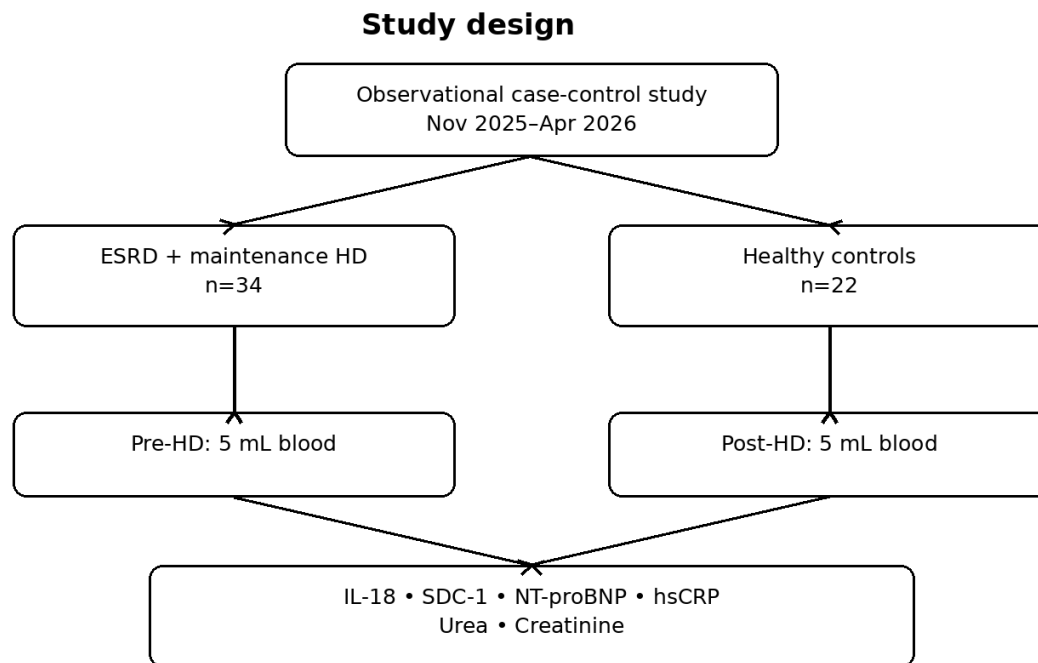
NT-proBNP represents a complementary cardiovascular domain. It is released in response to myocardial wall stress and is widely used in the assessment of cardiac strain. In ESRD, however, its interpretation is confounded by reduced renal clearance (Adams et al., 1994), chronic volume overload and treatment-related alterations in plasma volume. Reviews note that NT-proBNP in dialysis represents a blend of cardiac and renal effects, rather than cardiac dysfunction specifically (Chaikijurajai et al., 2020). A systematic review and meta-analysis likewise identified the impact of hemodialysis on NT-proBNP to be heterogeneous, being dependent on treatment characteristics (Yin et al., 2021).

Thus, concurrent measurement of IL-18, hsCRP, SDC -1 and both NT-proBNP and TNF- $\alpha$  gene expression may yield a more unifying view—across the protein and transcriptional levels—of the biological impact of ESRD/dialysis. The aims of the present study were to ascertain if these potential biomarkers are different in ESRD patients compared to healthy controls, and whether they change acutely by investigating their pre/post-dialysis levels, and their interrelationships with urea and creatinine; as well as application as discriminant between dialysis patients and healthy individuals.

## 2. MATERIALS AND METHODS

### 2.1 Study design and participants

A paired pre- and post-hemodialysis observational case-control study was performed in the Dialysis Unit of Imam Sadiq Teaching Hospital (AS) between Nov 2025 and April 2026. Methods: 54 participants were included in the study, comprising 34 patients with end-stage renal disease (ESRD) and 22 apparently healthy controls. Patients were aged 18–60 years, clinically stable and on regular maintenance hemodialysis; controls had no evidence of chronic or acute systemic disease. Convenience sampling was used, and no prospective formal power calculation was performed.



**Figure 1. Schematic representation of the study design.**

## 2.2 Ethical approval and eligibility

Ethical approval was obtained from the Research Ethics Committee of Al-Qasim Green University (Certificate No. qgec/62/2026), approved 23 January 2026 and valid until 23 January 2027. Inclusion criteria were adulthood, confirmed ESRD, regular maintenance hemodialysis, clinical stability and written informed consent. Exclusion criteria included acute renal failure, active infection/inflammation, autoimmune disease, malignancy, liver disease, recent surgery, pregnancy, corticosteroid therapy and recent acute cardiovascular events.

## 2.3 Hemodialysis and sample collection

Patients underwent four-hour high-flux hemodialysis using a NIPRO Surdial 55Plus machine. Blood flow was approximately 250–300 mL/min and dialysate flow 500 mL/min; ultrafiltration was individualized according to interdialytic weight gain and clinical assessment. Blood samples (5 mL) were collected immediately before and after the same dialysis session. Pre-HD samples were obtained before heparin administration. For biomarker ELISA, serum was separated after 30 minutes of clotting by centrifugation at 3500 rpm for 7 minutes and stored at  $-20^{\circ}\text{C}$  until analysis. A parallel 2.5 mL aliquot of whole blood was collected into EDTA tubes containing an RNA-stabilizing reagent for downstream gene-expression analysis.

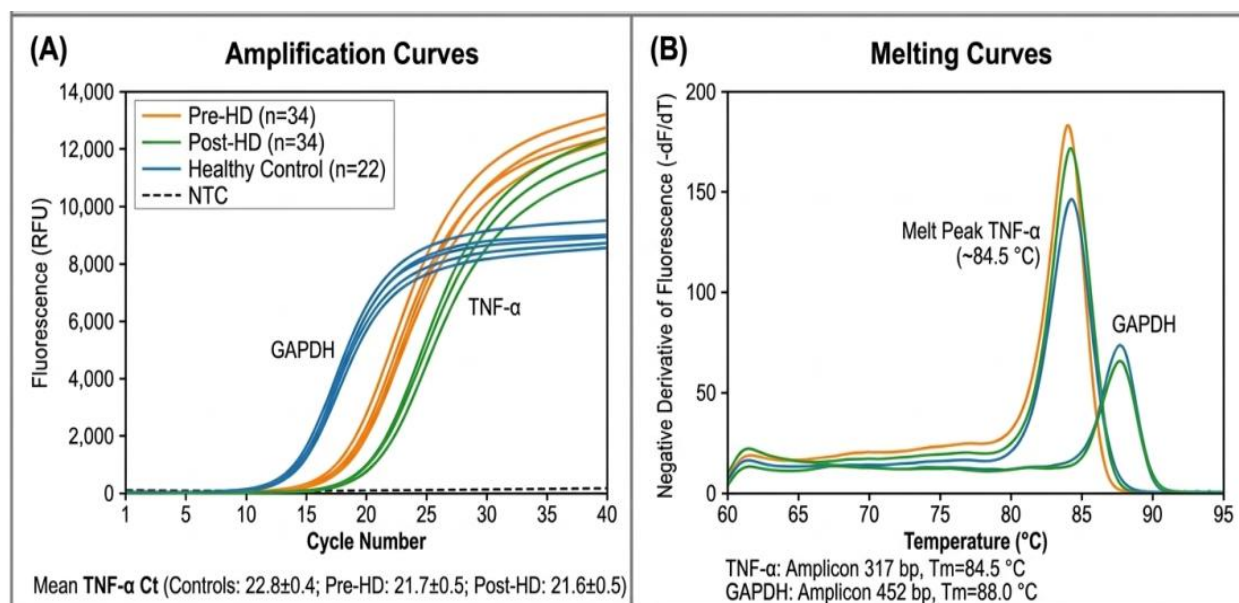
## 2.4 Laboratory assays of protein biomarkers

Urea was measured by an enzymatic urease–glutamate dehydrogenase colorimetric method and creatinine by an enzymatic colorimetric assay. IL-18, SDC-1, NT-proBNP and hsCRP were measured using sandwich ELISA kits supplied by Bioassay Technology Laboratory (BT LAB), China. The reported kit detection ranges were 0.5–100 ng/L for IL-18 and 0.1–40 ng/mL for SDC-1, NT-proBNP and hsCRP. ELISA signal was read at 450 nm and concentrations were calculated from standard curves. Instruments were calibrated and assay procedures followed the manufacturer's instructions.

## 2.5 RNA extraction, reverse transcription and quantitative real-time PCR analysis of TNF- $\alpha$ gene expression

Total RNA was extracted from whole-blood leukocytes using a commercial column-based RNA extraction kit according to the manufacturer's protocol. RNA concentration and purity were verified spectrophotometrically (A260/A280 ratio 1.8–2.1) before complementary DNA (cDNA) synthesis by reverse transcription. Quantitative real-time PCR (RT-qPCR) was performed in duplicate for TNF- $\alpha$  and for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH), which served as the internal reference gene for normalization. Primer sequences of TNF- $\alpha$  F: CCAAGGGAGAGTGGTCAGGT / R: GGCAACAAGGTAGAGAGGC and GAPDH gene were F: ACCACAGTCCATGCCATCAC / R: TCCACCACCTGTTGCTGTA and the Amplicon length (bp) were 317 for TNF- $\alpha$  gene and 452 for GAPDH gene.

Amplification was carried out on a real-time thermal cycler using a SYBR-Green-based master mix, and reporting followed the updated Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE 2.0) framework (Bustin et al., 2025). Primer amplification efficiencies were determined from standard-curve dilution series and were 1.95 for TNF- $\alpha$  and 2.00 for GAPDH (Figure 2).



**Figure 2: Analysis of TNF- $\alpha$  gene expression by Quantitative Real-time PCR technique (Human Peripheral Blood). Demonstration of Amplification curves (A) and melting curves (B). The TNF- $\alpha$  (traces crossing cycle 22) and GAPDH (traces crossing cycle 18) data are shown as smooth, colour-coded traces representing duplicated samples. A key indicates: Pre-HD (n=34), Post-HD (n=34), Healthy Control (n=22) and NTCs (No Template Controls, flat). Text below the graph shows average cycle threshold (Ct) values: Mean TNF- $\alpha$  Ct (Controls: 22.8±0.4; Pre-HD: 21.7±0.5; Post-HD: 21.6±0.5). Solid, color-coded traces indicate single sharp melting peaks for the three groups; the TNF- $\alpha$  target melt peak is labeled: Melt Peak TNF- $\alpha$  (~84.5 °C). Data are given as average melting temperatures: TNF- $\alpha$  T<sub>m</sub>=84.5 °C, GAPDH T<sub>m</sub>=88.0 °C; TNF- $\alpha$  mRNA expression (fold-change vs control mean) is significantly upregulated in Pre-HD (2.03-fold), Post-HD (2.07-fold) vs Healthy Controls (1.00-fold); none of the paired changes after a single high-flux hemodialysis session were significant (P=0.723), consistent with large AUC values (0.95–0.96) to discriminate patients from controls (MIQE 2.0 framework, primers efficiency-corrected 1.95–2.00).**

## 2.6 Statistical analysis

SPSS version 27 was used for the protein biomarker and clinical data; TNF- $\alpha$  gene-expression data were additionally analyzed in Python (SciPy 1.17) to allow ROC bootstrapping. Normality was assessed using the Shapiro–Wilk test. Mann–Whitney U tests were used for independent comparisons, Wilcoxon signed-rank tests for paired pre/post comparisons, and Spearman correlation for associations. TNF- $\alpha$  relative expression values were approximately normally distributed (Shapiro–Wilk P>0.05 in all three groups); nonparametric tests were nevertheless retained for TNF- $\alpha$ , consistent with the analytical framework applied to the other biomarkers and appropriate given the modest sample size. ROC analysis assessed discrimination of HD patients from controls, with the optimal cut-off selected by the Youden index and 95% confidence intervals for the area under the curve (AUC) obtained by bootstrap resampling (2,000 replicates). P<0.05 was considered statistically significant. Results were expressed as mean±SD or median (IQR), according to distribution.

## 3. RESULTS

### 3.1 Baseline characteristics

**Table 1. Baseline demographic and clinical characteristics.**

| Variable                | HD (n=34)    | Controls (n=22) | P      |
|-------------------------|--------------|-----------------|--------|
| Age, years              | 48.85±12.79  | 42.50±15.19     | 0.098  |
| Male                    | 21 (61.8%)   | 8 (36.4%)       | 0.063  |
| Height, cm              | 168.50±10.20 | 166.14±9.26     | 0.384  |
| Weight, kg              | 70.38±11.94  | 70.18±10.66     | 0.949  |
| BMI, kg/m <sup>2</sup>  | 24.83±4.03   | 25.60±3.73      | 0.475  |
| Hypertension            | 15 (44.1%)   | 0               | 0.028* |
| Diabetes + hypertension | 15 (44.1%)   | 0               | —      |
| Previous COVID-19       | 13 (38.2%)   | 8 (36.4%)       | 0.888  |

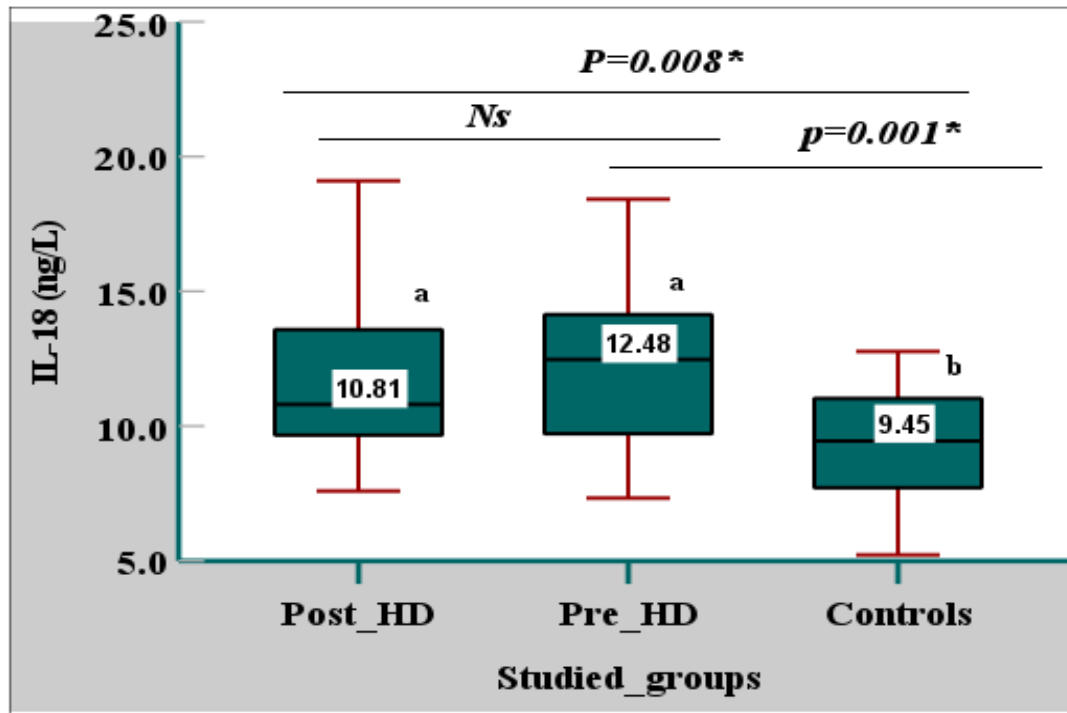
The groups were relatively similar in age, anthropometry and previous history of COVID-19. The primary baseline difference was comorbidity burden, with hypertension and combined diabetes/hypertension present in the HD but not control group.

### 3.2 Controls versus Pre-HD

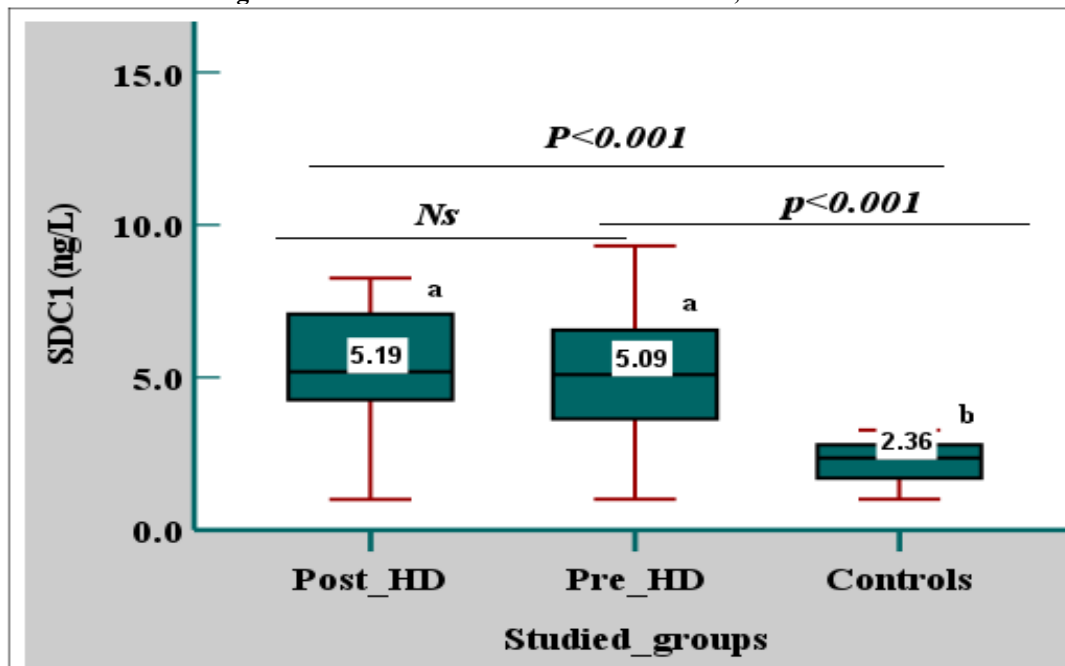
**Table 2. Biomarkers and renal parameters in controls and Pre-HD patients.**

| Variable          | Control median (IQR) | Pre-HD median (IQR) | P       |
|-------------------|----------------------|---------------------|---------|
| IL-18, ng/L       | 9.45 (7.66–11.05)    | 12.48 (9.66–14.19)  | 0.001*  |
| SDC-1, ng/L       | 2.36 (1.69–2.83)     | 5.09 (3.61–6.66)    | <0.001* |
| NT-proBNP, ng/L   | 3.67 (2.28–4.98)     | 9.30 (8.08–9.98)    | <0.001* |
| hsCRP, ng/L       | 2.32 (1.57–3.69)     | 5.30 (4.84–6.19)    | <0.001* |
| Urea, mg/dL       | 5.00 (4.00–7.00)     | 41.00 (30.00–52.00) | <0.001* |
| Creatinine, mg/dL | 0.50 (0.40–0.70)     | 11.00 (7.75–15.50)  | <0.001* |

The levels of all four protein biomarkers were significantly higher before dialysis compared to controls, although the difference in SDC-1 between the two groups was larger. Elevated urea and creatinine level, confirming on max lessened renal clearance typical of ESRD (Figures 3-7).



**Figure 3. Serum IL-18 distribution in Pre-HD, Post-HD and controls.**



**Figure 4. Serum SDC-1 distribution in Pre-HD, Post-HD and controls.**

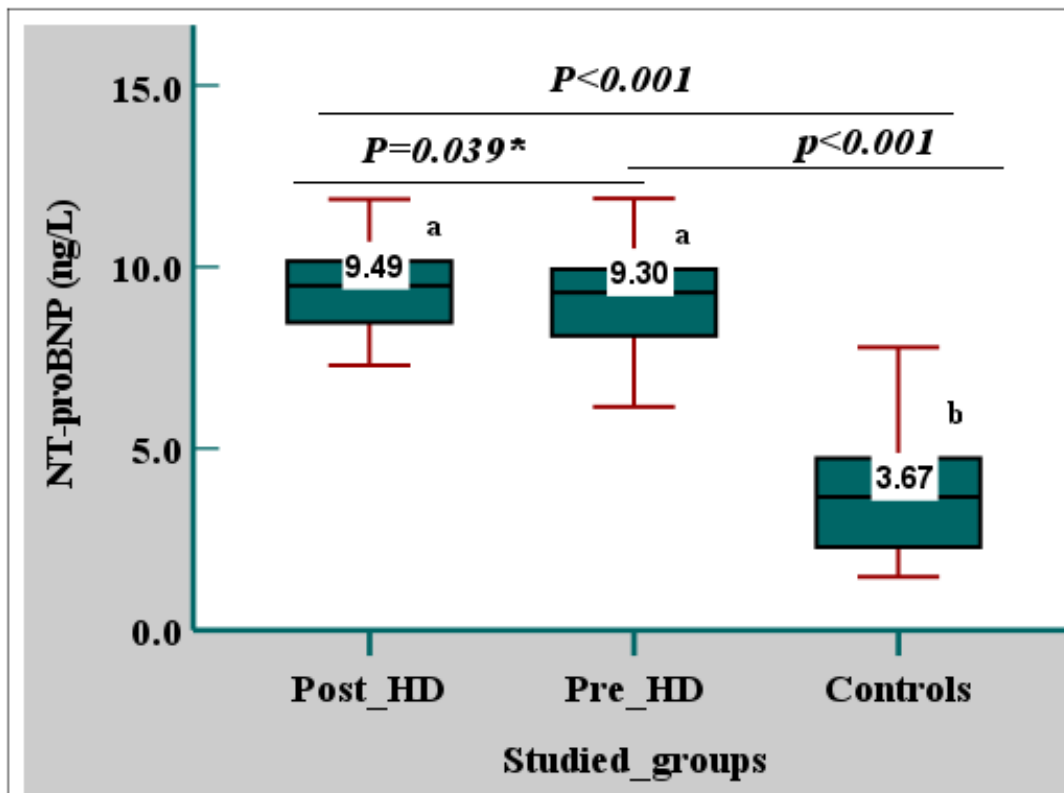


Figure 5. Serum NT-proBNP distribution in Pre-HD, Post-HD and controls.

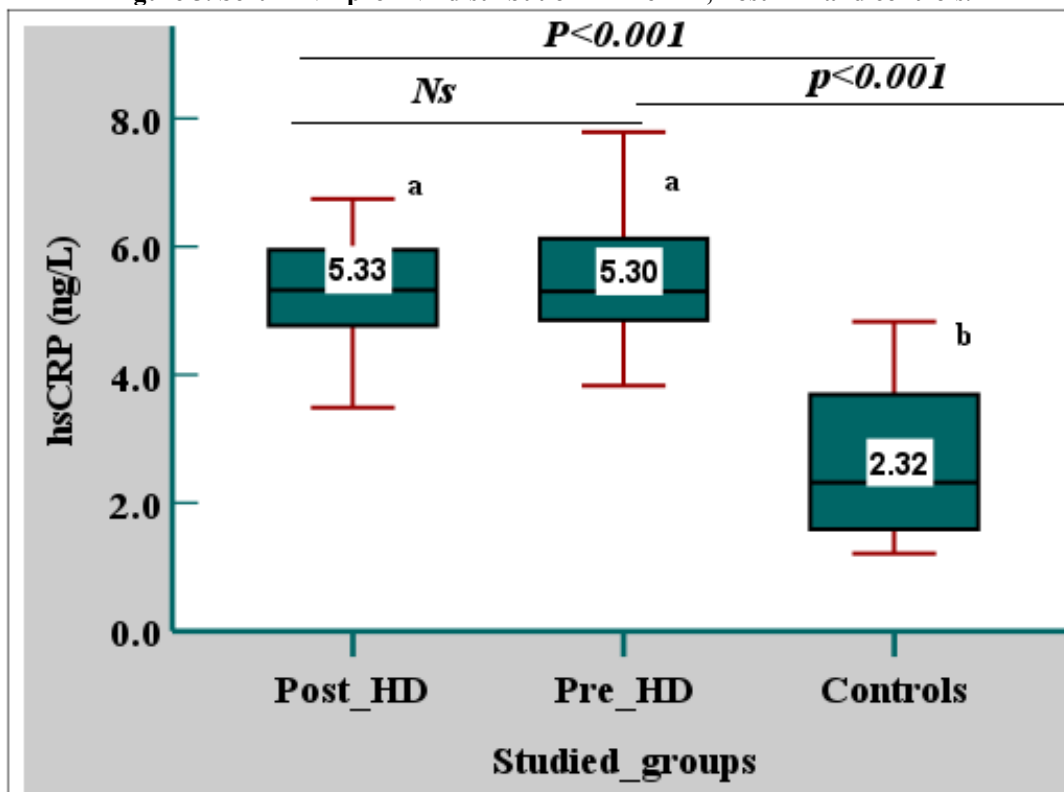


Figure 6. Serum hsCRP distribution in Pre-HD, Post-HD and controls.

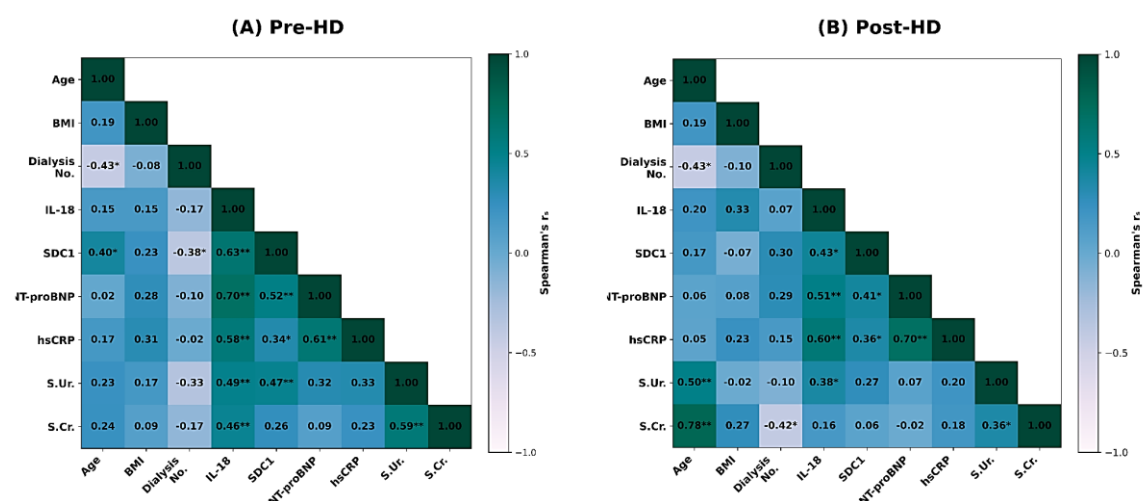


Figure 7. Spearman correlation heatmaps for Pre-HD and Post-HD groups.

### 3.3 Controls versus Post-HD

Table 3. Biomarkers and renal parameters in controls and Post-HD patients.

| Variable          | Control median (IQR) | Post-HD median (IQR) | P       |
|-------------------|----------------------|----------------------|---------|
| IL-18, ng/L       | 9.45 (7.66–11.05)    | 10.81 (9.55–13.62)   | 0.008*  |
| SDC-1, ng/L       | 2.36 (1.69–2.83)     | 5.19 (4.22–7.15)     | <0.001* |
| NT-proBNP, ng/L   | 3.67 (2.28–4.98)     | 9.49 (8.42–10.17)    | <0.001* |
| hsCRP, ng/L       | 2.32 (1.57–3.69)     | 5.33 (4.72–5.98)     | <0.001* |
| Urea, mg/dL       | 5.00 (4.00–7.00)     | 18.00 (16.00–21.25)  | <0.001* |
| Creatinine, mg/dL | 0.50 (0.40–0.70)     | 3.68 (2.96–5.35)     | <0.001* |

All four protein biomarkers remained significantly higher after dialysis than in controls. Urea and creatinine were greatly reduced relative to the pre-HD state but remained above control values, demonstrating residual azotemia despite dialysis.

### 3.4 Paired Pre-HD versus Post-HD

Table 4. Paired changes following one hemodialysis session.

| Variable          | Pre-HD mean±SD | Post-HD mean±SD | P       |
|-------------------|----------------|-----------------|---------|
| IL-18, ng/L       | 13.70±6.50     | 12.80±5.35      | 0.334   |
| SDC-1, ng/L       | 5.74±4.22      | 6.29±4.20       | 0.626   |
| NT-proBNP, ng/L   | 9.79±3.02      | 10.34±3.49      | 0.039*  |
| hsCRP, ng/L       | 6.32±3.14      | 6.27±3.52       | 0.765   |
| Urea, mg/dL       | 41.94±14.04    | 18.62±3.33      | <0.001* |
| Creatinine, mg/dL | 12.18±5.69     | 4.52±2.44       | <0.001* |

Urea and creatinine fell sharply within the same dialysis session, confirming effective small-solute clearance. IL-18, SDC-1 and hsCRP did not change significantly, whereas NT-proBNP increased modestly but significantly, consistent with treatment-related hemoconcentration and impaired clearance rather than a uniform biomarker response to dialysis.

### 3.5 Dialysis session-frequency subgroups

Table 5. Biomarkers and renal parameters by dialysis session-frequency category (≤4 vs. >4 sessions).

| Variable       | ≤4 sessions median (IQR) | >4 sessions median (IQR) | P     |
|----------------|--------------------------|--------------------------|-------|
| IL-18 Pre      | 12.60 (9.98–14.05)       | 11.90 (9.20–14.85)       | 0.842 |
| SDC-1 Pre      | 5.76 (3.98–6.98)         | 4.63 (2.44–6.51)         | 0.312 |
| SDC-1 Post     | 4.72 (3.56–6.81)         | 5.43 (4.78–7.21)         | 0.107 |
| NT-proBNP Pre  | 9.48 (7.38–9.89)         | 9.17 (8.21–10.32)        | 0.607 |
| NT-proBNP Post | 9.50 (7.52–9.97)         | 9.48 (8.83–10.62)        | 0.264 |
| hsCRP Pre      | 5.09 (4.56–6.06)         | 5.39 (4.87–6.32)         | 0.208 |
| hsCRP Post     | 5.33 (4.26–6.20)         | 5.32 (4.87–5.77)         | 0.818 |
| Urea Pre       | 43.00 (32.00–56.00)      | 38.00 (29.50–45.50)      | 0.256 |
| Creatinine Pre | 12.00 (7.50–19.50)       | 10.00 (7.50–14.00)       | 0.606 |

No significant differences were observed between the  $\leq 4$  and  $> 4$  session categories for the reported biomarkers or renal parameters. Thus, in this cohort, session frequency alone did not explain biomarker variability.

### 3.6 Correlation analysis

Before dialysis, IL-18 correlated positively with NT-proBNP ( $r=0.70$ ,  $P<0.01$ ), SDC-1 ( $r=0.63$ ,  $P<0.01$ ), hsCRP ( $r=0.58$ ,  $P<0.01$ ), urea ( $r=0.49$ ,  $P<0.01$ ) and creatinine ( $r=0.46$ ,  $P<0.01$ ). NT-proBNP correlated with hsCRP ( $r=0.61$ ,  $P<0.01$ ). SDC-1 correlated positively with age ( $r=0.40$ ,  $P<0.05$ ) and inversely with dialysis-session number ( $r=-0.38$ ,  $P<0.05$ ).

After dialysis, IL-18 correlated with hsCRP ( $r=0.60$ ,  $P<0.01$ ), NT-proBNP ( $r=0.51$ ,  $P<0.01$ ) and SDC-1 ( $r=0.43$ ,  $P<0.05$ ). NT-proBNP and hsCRP showed the strongest post-HD biomarker correlation ( $r=0.70$ ,  $P<0.01$ ). Age correlated strongly with creatinine ( $r=0.78$ ,  $P<0.01$ ) and urea ( $r=0.50$ ,  $P<0.01$ ). Patient-level linkage between the TNF- $\alpha$  gene-expression dataset and this previously established protein biomarker panel was not available; consequently, the relationship between TNF- $\alpha$  transcription and the protein markers is addressed at the group level (Sections 3.7–3.8 and Discussion) rather than through individual-patient correlation coefficients.

### 3.7 ROC analysis of protein biomarkers

**Table 6. Diagnostic performance of protein biomarkers for discriminating HD patients from healthy controls.**

| Biomarker | Pre-HD AUC       | Cut-off      | Se     | Sp     | Post-HD AUC      | Se     | Sp    |
|-----------|------------------|--------------|--------|--------|------------------|--------|-------|
| IL-18     | 0.77 (0.65–0.89) | $\geq 12.79$ | 50.0%  | 100.0% | 0.71 (0.58–0.85) | 67.6%  | 63.6% |
| SDC-1     | 0.83 (0.71–0.95) | $\geq 3.08$  | 85.3%  | 86.4%  | 0.87 (0.76–0.98) | 91.2%  | 90.9% |
| NT-proBNP | 0.97 (0.92–1.00) | $\geq 5.94$  | 100.0% | 81.8%  | 0.98 (0.95–1.00) | 100.0% | 86.4% |
| hsCRP     | 0.98 (0.96–1.00) | $\geq 4.36$  | 94.1%  | 95.5%  | 0.97 (0.93–1.00) | 91.2%  | 90.9% |

All four biomarkers had statistically significant discrimination. hsCRP and NT-proBNP showed the highest AUC values, SDC-1 showed good discrimination, and IL-18 showed moderate discrimination. These values describe separation from the selected healthy controls and should not be interpreted as disease-specific clinical thresholds.

### 3.8 TNF- $\alpha$ gene expression analysis

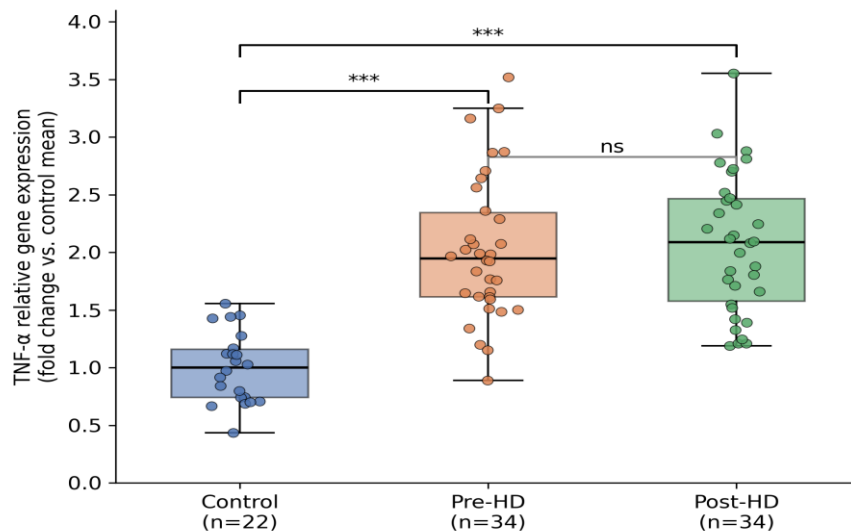
Peripheral blood TNF- $\alpha$  mRNA expression, normalized to GAPDH and expressed as fold-change relative to the mean control value, was significantly higher in ESRD patients than in controls both before and after dialysis (Table 7, Figure 8). Control subjects showed a mean relative expression of  $1.00 \pm 0.30$ -fold (median 1.00, IQR 0.74–1.16), consistent with the normalization of the group to its own mean. Pre-HD patients showed a mean relative expression of  $2.03 \pm 0.62$ -fold (median 1.95, IQR 1.61–2.34), representing an approximately two-fold up-regulation relative to controls (Mann–Whitney  $U=717$ ,  $P<0.001$ ; Cohen's  $d=1.96$ , a large effect size). Post-HD patients showed a mean relative expression of  $2.07 \pm 0.60$ -fold (median 2.09, IQR 1.58–2.47), also approximately two-fold higher than controls (Mann–Whitney  $U=714$ ,  $P<0.001$ ; Cohen's  $d=2.11$ ).

In the paired within-patient analysis, TNF- $\alpha$  relative expression did not change significantly across the single dialysis session (Pre-HD  $2.03 \pm 0.62$ -fold vs. Post-HD  $2.07 \pm 0.60$ -fold; Wilcoxon signed-rank  $W=276$ ,  $P=0.723$ ), a post/pre fold-ratio of 1.02. This pattern — marked up-regulation relative to controls with no significant acute change across one dialysis session — closely parallels the behavior of hsCRP, IL-18 and SDC-1 described in Sections 3.2–3.4, and stands in contrast to the rapid, dialysis-responsive clearance of urea and creatinine.

**Table 7. TNF- $\alpha$  relative gene expression (fold-change vs. control mean) in controls, Pre-HD and Post-HD patients.**

| Group   | n  | Mean $\pm$ SD   | Median (IQR)     | P vs. Control |
|---------|----|-----------------|------------------|---------------|
| Control | 22 | $1.00 \pm 0.30$ | 1.00 (0.74–1.16) | —             |
| Pre-HD  | 34 | $2.03 \pm 0.62$ | 1.95 (1.61–2.34) | $<0.001^*$    |
| Post-HD | 34 | $2.07 \pm 0.60$ | 2.09 (1.58–2.47) | $<0.001^*$    |

Paired Pre-HD vs. Post-HD comparison: Wilcoxon signed-rank  $W=276$ ,  $P=0.723$  (not significant). Fold-change Pre-HD vs. control = 2.03; Post-HD vs. control = 2.07; Post-HD/Pre-HD ratio = 1.02.



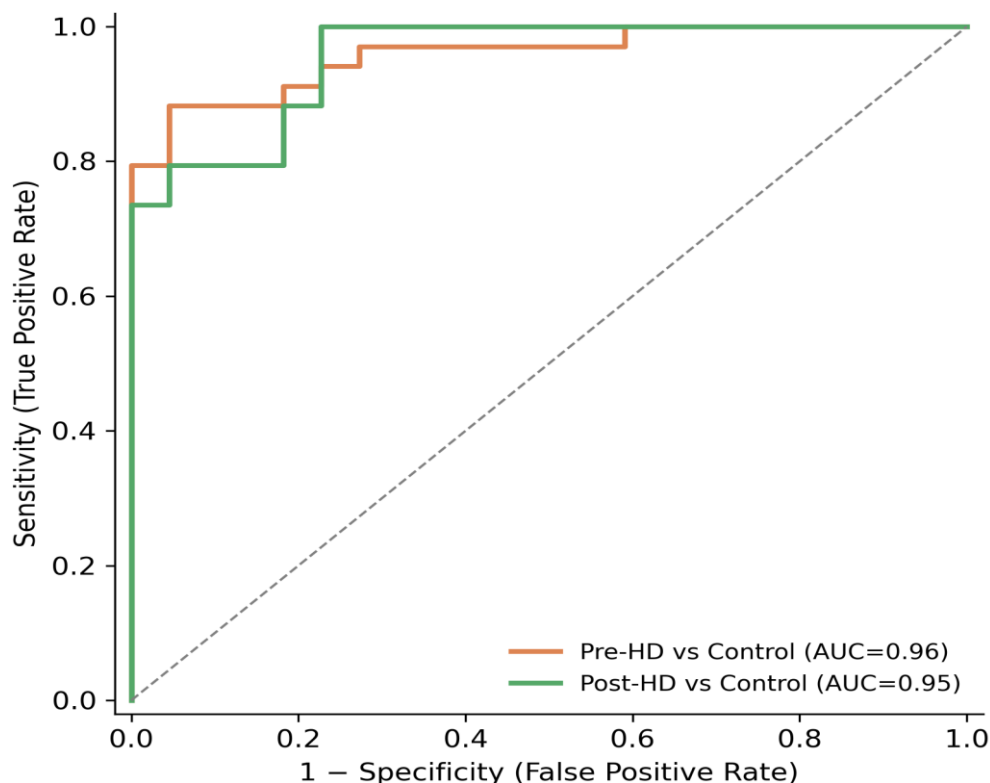
**Figure 8. TNF- $\alpha$  relative gene expression (fold-change vs. control mean) in Control, Pre-HD and Post-HD groups. Boxes show median and interquartile range; points show individual samples. \*\*\*P<0.001 vs. control (Mann–Whitney U); ns = not significant, Pre-HD vs. Post-HD (Wilcoxon signed-rank).**

ROC analysis of TNF- $\alpha$  relative gene expression showed excellent discrimination of ESRD patients from healthy controls both before and after dialysis (Table 8, Figure 9), performance comparable to that of SDC-1 and only modestly lower than hsCRP and NT-proBNP (Table 6).

**Table 8. Diagnostic performance of TNF- $\alpha$  gene expression for discriminating HD patients from healthy controls.**

| Comparison          | AUC (95% CI)     | Cut-off (fold) | Se     | Sp    |
|---------------------|------------------|----------------|--------|-------|
| Pre-HD vs. Control  | 0.96 (0.91–0.99) | $\geq 1.49$    | 88.2%  | 95.5% |
| Post-HD vs. Control | 0.95 (0.90–0.99) | $\geq 1.19$    | 100.0% | 77.3% |

*Cut-offs were selected by the Youden index; 95% confidence intervals were obtained by bootstrap resampling (2,000 replicates).*



**Figure 9. Receiver-operating-characteristic curves for TNF- $\alpha$  relative gene expression discriminating Pre-HD (orange) and Post-HD (green) patients from healthy controls.**

#### 4. DISCUSSION

The principal finding of this study is that hemodialysis produced a marked biochemical effect on conventional renal indices but a limited acute effect on the inflammatory, endothelial and transcriptional biomarkers examined. Urea and creatinine fell substantially, confirming effective small-solute clearance, whereas IL-18, SDC-1, hsCRP and TNF- $\alpha$  gene expression remained statistically stable across the dialysis session. NT-proBNP increased modestly. Such discrepancies highlight that dialysis clearance of small solutes does not immediately reverse underlying chronic biological processes, in particular, the inflammatory transcriptional program associated with ESRD.

Urea and creatinine pre-HD concentrations >90 mM and <1000  $\mu$ M/L upon initiation of maintenance dialysis, respectively, are physiologically expected values that also allow for useful internal references of treatment efficacy. Their relatively large post-HD decrease in cytokines is consistent with diffusion and convection across the high-flux membrane. Nonetheless both remained elevated well above values seen in unmedicated healthy-controls. Dialysis is an intermittent treatment and therefore can only reduce but not reproduce the continuous clearance provided by functioning kidneys. Hence this residual biochemical difference is consistent with chronic kidney disease rather than inadequate dialysis as the only mechanism. The increase hsCRP levels observed before and after dialysis are consistent with the established inflammatory phenotype of chronic dialysis. Nowak and Chonchol (2018) emphasized that inflammation in dialysis is multifactorial, involving the uremic environment, comorbid conditions, infection, dysbiosis and treatment-related exposures, and that increased inflammatory markers are associated with adverse clinical outcomes. Antunović et al. (2017) similarly showed that hsCRP independently predicted both all-cause and cardiovascular mortality over two years of follow-up in hemodialysis patients. The absence of a significant paired reduction in hsCRP in the present study is therefore plausible because a single treatment session is unlikely to eliminate a chronic inflammatory state.

IL-18 showed a similar pattern. Its pre-HD elevation and persistence after dialysis support its role as a marker of chronic inflammatory activation. IL-18 is mechanistically linked to innate immune signaling and inflammatory kidney disease (Hirooka & Nozaki, 2021). The present positive correlations between IL-18 and urea and creatinine suggest that greater uremic burden may accompany stronger inflammatory activation. The relationship should, however, be interpreted as an association rather than proof that uremic solutes directly cause IL-18 elevation.

The TNF- $\alpha$  gene-expression findings extend this inflammatory narrative to the transcriptional level. TNF- $\alpha$  mRNA was approximately two-fold up-regulated in ESRD patients relative to controls both before and after dialysis, with large effect sizes (Cohen's *d* 1.96 and 2.11, respectively) and excellent discrimination on ROC analysis (AUC 0.96 and 0.95). This pattern is consistent with mechanistic and genetic evidence implicating TNF- $\alpha$  signaling, via TNF receptors 1 and 2, in the pathogenesis and progression of CKD (Lousa et al., 2022), with experimental data showing that TNF- $\alpha$  inhibition attenuates renal inflammatory gene expression and fibrosis (Taguchi et al., 2021), and with genetic association data linking TNF- $\alpha$  promoter polymorphisms to ESRD susceptibility (Anumas et al., 2025). The persistence of TNF- $\alpha$  up-regulation after a single dialysis session, mirroring the pattern of hsCRP, IL-18 and SDC-1, suggests that circulating leukocyte TNF- $\alpha$  transcription reflects a relatively stable, dialysis-resistant inflammatory program rather than an acutely dialyzable factor, consistent with the broader observation that gene-expression-based inflammatory markers in hemodialysis patients tend to track chronic disease burden more closely than single-session treatment effects (Okuyan et al., 2023).

The SDC-1 findings provide evidence for a vascular component of ESRD. Median SDC-1 was more than twice as high in pre-HD patients as in controls and remained elevated after dialysis. This is consistent with earlier work demonstrating that circulating markers of endothelial glycocalyx damage, including syndecan-1, are elevated in dialysis patients and correlate with uremic toxin burden and endothelial dysfunction markers (Liew et al., 2021). The lack of a statistically significant pre/post change is nevertheless important because published studies have reported both stable and increased syndecan-1 during dialysis. Koch et al. (2020) showed that syndecan-1 can increase during treatment in association with acute plasma sodium changes, and Kusuzawa et al. (2021) documented a significant pre/post increase in a 145-patient cohort.

The discrepancy between the present paired result and some published cohorts may reflect differences in membrane characteristics, anticoagulation, sodium exposure, ultrafiltration, treatment duration, patient comorbidity and sampling time. The current study collected only two samples, immediately before and after dialysis, so transient intradialytic peaks could have been missed. The numerically higher post-HD mean SDC-1 nevertheless points in the same biological direction as studies demonstrating dialysis-associated glycocalyx shedding, even though the change did not reach statistical significance.

NT-proBNP behaved differently from the other biomarkers, increasing significantly after dialysis. This result should not be interpreted automatically as evidence of acute myocardial injury. NT-proBNP is strongly influenced by renal clearance and volume status in ESRD (Chaikijurajai et al., 2020). Hemodialysis can change plasma volume and concentration, while the underlying renal impairment persists. A systematic review found considerable heterogeneity in the pre/post dialysis response of NT-proBNP (Yin et al., 2021). Therefore, the modest increase in the present study is compatible with treatment-related hemoconcentration and persistent impaired clearance.

The correlation findings support an integrated pathophysiological model at the protein level. IL-18 was strongly associated with NT-proBNP, SDC-1 and hsCRP before dialysis, and these relationships persisted after treatment. This indicates that inflammation, endothelial injury and cardiac stress are not isolated abnormalities. Rather, they may be different manifestations of the same uremic and cardiovascular environment. Because patient-level linkage between the TNF- $\alpha$  gene-expression dataset and the protein biomarker panel was not available, a direct statistical correlation between TNF- $\alpha$  transcription and IL-18, SDC-1, NT-proBNP or hsCRP could not be computed in the present cohort; this is stated explicitly as a limitation rather than inferred. Nonetheless, the concordant group-level behavior of TNF- $\alpha$  expression and the protein markers pronounced elevation in ESRD relative to controls, and stability across a single dialysis session is compatible with a shared, TNF- $\alpha$ -linked inflammatory axis and provides a rational basis for prospective studies with paired, patient-level multi-omic sampling.

The strong post-HD relationship between NT-proBNP and hsCRP further suggests that cardiac stress and systemic inflammation remain linked even after correction of some uremic solute abnormalities. This is consistent with the concept that cardiovascular disease in ESRD is not simply a consequence of volume overload. Chronic inflammation, endothelial dysfunction, vascular calcification, oxidative stress, altered myocardial loading and, plausibly, sustained TNF- $\alpha$ -driven transcriptional activation all contribute to cardiovascular risk. The present biomarker pattern cannot establish the direction of these relationships, but it provides a coherent biological signal that spans the protein and gene-expression levels.

The absence of significant biomarker differences between the low- and high-session-frequency groups should also be interpreted carefully. Session frequency is only one component of dialysis exposure. Dialysis adequacy, ultrafiltration rate, residual kidney function, membrane properties, dialysis vintage, nutritional status and comorbidity may be more important determinants of circulating and transcriptional biomarker concentrations. The small sample size and categorical grouping used here may also have limited statistical power to detect modest effects.

The ROC results were striking for hsCRP, NT-proBNP and TNF- $\alpha$  gene expression. Pre-HD AUCs were 0.98 (hsCRP), 0.97 (NT-proBNP) and 0.96 (TNF- $\alpha$ ), while post-HD AUCs were 0.97, 0.98 and 0.95, respectively. SDC-1 showed good discrimination (AUC 0.83–0.87), whereas IL-18 showed lower but significant discrimination (AUC 0.71–0.77). These results suggest that the biomarkers, including TNF- $\alpha$  transcription, contain substantial information for distinguishing this ESRD cohort from healthy controls. However, the very high AUCs should not be generalized to real-world diagnostic discrimination between ESRD and other patient groups. The control group was apparently healthy, making the contrast physiologically large.

The NT-proBNP ROC result is biologically plausible but also demonstrates why renal context matters. Natriuretic peptides are affected by impaired renal clearance and volume status, so a high AUC against healthy individuals does not mean that NT-proBNP alone can distinguish cardiac disease from renal disease in a clinical population. Similarly, hsCRP is a sensitive inflammatory marker but is nonspecific, and TNF- $\alpha$  gene expression, while highly discriminative in this cohort, reflects a broadly activated inflammatory transcriptional state rather than a kidney-specific process. The diagnostic value of these biomarkers is therefore likely to be greatest when interpreted together with clinical status and conventional renal assessment.

SDC-1 provided additional discrimination and may be useful because it represents a biological domain not captured by urea, creatinine or hsCRP. The endothelial glycocalyx is increasingly recognized as an important component of vascular homeostasis, and dialysis patients have demonstrable glycocalyx damage (Liew et al., 2021; Kusuzawa et al., 2021). The present data extend this concept by showing that SDC-1 is substantially elevated in the local ESRD cohort and remains elevated immediately after dialysis.

IL-18 showed the lowest AUC values among the protein markers, but this does not mean that it lacks biological relevance. A biomarker may participate strongly in disease mechanisms yet overlap substantially between patients and controls. The strong correlations of IL-18 with other biomarkers in the present study suggest that it may be more useful as part of a multimarker panel than as a stand-alone diagnostic test. Recent ESRD research similarly supports the relevance of IL-18 to adverse outcomes and inflammatory burden (Liu et al., 2022). The consistency between group comparisons, paired analysis, correlation structure and ROC performance strengthens the overall interpretation: hemodialysis efficiently clears small solutes, but the inflammatory, endothelial, cardiovascular and transcriptional phenotype of ESRD persists beyond a single treatment session.

## 5. CONCLUSION

In this cohort, ESRD patients undergoing maintenance hemodialysis had significantly elevated IL-18, SDC-1, NT-proBNP and hsCRP compared with healthy controls, and peripheral blood TNF- $\alpha$  gene expression was similarly up-regulated approximately two-fold. A single high-flux dialysis session markedly reduced urea and creatinine but did not significantly change IL-18, SDC-1, hsCRP or TNF- $\alpha$  gene expression; NT-proBNP increased modestly. Strong positive relationships among inflammatory, endothelial and cardiac protein biomarkers indicate an interconnected pathophysiological phenotype, and the concordant behavior of TNF- $\alpha$  mRNA expression extends this phenotype to the transcriptional level. hsCRP, NT-proBNP and TNF- $\alpha$  gene expression showed the strongest discrimination between dialysis patients and healthy controls, while SDC-1 provided additional information and IL-18 showed moderate discrimination.

### Author Contribution

The authors (SMA, RSAA and KSM were equally responsible for study conception and design, experimental work, data collection, data analysis, and the manuscript preparation.

#### **Data availability statement**

All data that support the findings are available upon reasonable request.

#### **Acknowledgments**

The authors would like to express their sincere appreciation to the staff of college of science, Al- Qasim Green University, for their technical support and provision of laboratory facilities.

#### **Funding**

None.

#### **Ethical consideration**

All procedures involving human samples were approved by the Ethics Committee of the College of science, Al-Qasim Green University, Babylon, Iraq (approval no. qgec/44/2026).

#### **Conflict of interests**

The authors declare no competing interests related to this work.

## **6. REFERENCES**

1. Antunović, T., Stefanović, A., Gligorović Barhanović, N., Miljković, M., Radunović, D., Ivanišević, J., Prelević, V., Bulatović, N., & Ratković, M. (2017). Prooxidant–antioxidant balance, hsTnI and hsCRP: Mortality prediction in haemodialysis patients, two-year follow-up. *Renal Failure*, 39(1), 491–499. <https://doi.org/10.1080/0886022X.2017.1323645>
2. Anumas, S., Tansawet, A., Numthavaj, P., Pattharanitima, P., Pabalan, N., Jarjanazi, H., Mongkolrob, R., Tasanarong, A., & Tharabenjasin, P. (2025). Ethnicity-specific associations between the promoter region G-308A polymorphism (rs1800629) of the TNF- $\alpha$  gene and the development of end-stage renal disease: An evidence-based meta-analysis and trial sequential analysis. *Genetics and Molecular Biology*, 48(1), e20240077. <https://doi.org/10.1590/1678-4685-GMB-2024-0077>
3. Bustin, S. A., Ruijter, J. M., van den Hoff, M. J. B., Kubista, M., Pfaffl, M. W., Shipley, G. L., Tran, N., Rödiger, S., Untergasser, A., Mueller, R., Nolan, T., Milavec, M., Burns, M. J., Huggett, J. F., Vandesompele, J., & Wittwer, C. T. (2025). MIQE 2.0: Revision of the minimum information for publication of quantitative real-time PCR experiments guidelines. *Clinical Chemistry*, 71(6), 634–651. <https://doi.org/10.1093/clinchem/hvaf043>
4. Chaikijurajai, T., Rincon Choles, H., & Tang, W. H. W. (2020). Do natriuretic peptide measurements provide insights into management of end-stage renal disease patients undergoing dialysis? *Current Heart Failure Reports*, 17(6), 449–456. <https://doi.org/10.1007/s11897-020-00488-6>
5. Hirooka, Y., & Nozaki, Y. (2021). Interleukin-18 in inflammatory kidney disease. *Frontiers in Medicine*, 8, 639103. <https://doi.org/10.3389/fmed.2021.639103>
6. Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group. (2024). KDIGO 2024 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney International*, 105(4, Suppl.), S117–S314. <https://doi.org/10.1016/j.kint.2023.10.018>
7. Koch, J., Idzerda, N. M. A., Ettema, E. M., Dam, W. A., Meloen, M., Ellenbroek, J. H., van Beek, A. P., Nauta, F. L., Berger, S. P., Franssen, C. F. M., & Bakker, S. J. L. (2020). An acute rise of plasma Na<sup>+</sup> concentration associates with syndecan-1 shedding during hemodialysis. *American Journal of Physiology-Renal Physiology*, 319(2), F171–F177. <https://doi.org/10.1152/ajprenal.00005.2020>
8. Kooman, J. P., Katzarski, K., van der Sande, F. M., Leunissen, K. M. L., & Kotanko, P. (2018). Hemodialysis: A model for extreme physiology in a vulnerable patient population. *Seminars in Dialysis*, 31(5), 500–506. <https://doi.org/10.1111/sdi.12704>
9. Kusuzawa, K., Suzuki, K., Okada, H., Nakamura, N., Ushigome, E., Yamaguchi, T., Kotani, K., & Yoshida, S. (2021). Measuring the concentration of serum syndecan-1 to assess vascular endothelial glycocalyx injury during hemodialysis. *Frontiers in Medicine*, 8, 791309. <https://doi.org/10.3389/fmed.2021.791309>
10. Liew, H., Roberts, M. A., Pope, A., & McMahon, L. P. (2021). Endothelial glycocalyx damage in kidney disease correlates with uraemic toxins and endothelial dysfunction. *BMC Nephrology*, 22, 21. <https://doi.org/10.1186/s12882-020-02219-4>
11. Liu, Y. H., Chen, Y. H., Ko, C. H., Chen, H. Y., Chuang, Y. F., Tsai, Y. C., & Lin, C. Y. (2022). SOD3 and IL-18 predict the first kidney disease-related hospitalization or death during the one-year follow-up period in patients with end-stage renal disease. *Antioxidants*, 11(6), 1198. <https://doi.org/10.3390/antiox11061198>
12. Lousa, I., Reis, F., Santos-Silva, A., & Belo, L. (2022). The signaling pathway of TNF receptors: Linking animal models of renal disease to human CKD. *International Journal of Molecular Sciences*, 23(6), 3284. <https://doi.org/10.3390/ijms23063284>
13. Nowak, K. L., & Chonchol, M. (2018). Does inflammation affect outcomes in dialysis patients? *Seminars in Dialysis*, 31(4), 388–397. <https://doi.org/10.1111/sdi.12686>
14. Okuyan, H. M., Terzi, M. Y., Doğan, S., Emir, T., & Turgut, F. H. (2023). Association of miR-21-5p with routine biochemical markers and inflammatory cytokines in hemodialysis patients. *Gene Reports*, 31, 101761. <https://doi.org/10.1016/j.genrep.2023.101761>

15. Taguchi, S., Azushima, K., Yamaji, T., Urate, S., Suzuki, T., Abe, E., Tanaka, S., Tsukamoto, S., Kamimura, D., Kinguchi, S., Yamashita, A., Wakui, H., & Tamura, K. (2021). Effects of tumor necrosis factor- $\alpha$  inhibition on kidney fibrosis and inflammation in a mouse model of aristolochic acid nephropathy. *Scientific Reports*, 11, 23587. <https://doi.org/10.1038/s41598-021-02864-1>
16. Yin, L., Han, Z., Zhang, Q., Xie, J., Wang, C., & Zhao, L. (2021). The effect of hemodialysis on N-terminal pro-brain natriuretic peptide: A systematic review and meta-analysis. *American Journal of Emergency Medicine*, 44, 244–249. <https://doi.org/10.1016/j.ajem.2020.03.056>