

Molecular Insights into gene expression changes in apoptosis immune response and cellular regulation pathways in renal failure patients

Mustafa Riyadh Abdullah

Mustansiriyah University

Sahar Saadi Karieb

University of Baghdad

Omar Sinan Sadiq Hussain Al-Zaidi

omar.s94@uomustansiriyah.edu.iq

Mustansiriyah University

Research Article

Keywords: Chronic Kidney Disease, Caspase, PDCD1, CTLA4, TGFB3, Gene Expression

Posted Date: August 25th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10579802/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Abstract

Background: Chronic kidney disease (CKD) is a complex, multifactorial disease characterized by the overlapping pathways of apoptosis, immune regulation, and fibrosis, which play a pivotal role in disease progression. However, the molecular integration of these pathways remains incompletely understood, particularly regarding their combined impact on the deterioration of renal function.

Methods: The study included 60 participants: 30 patients with CKD and 30 healthy individuals as a control group. Hematological and biochemical parameters, including hemoglobin, hematocrit, urea, creatinine, and glucose, were assessed. Gene expression of CASP8, CASP9, PDCD1, CTLA4, and TGFB3 was analyzed using RT-qPCR, with the $2^{-\Delta\Delta C_t}$ method employed to calculate relative variability. Statistical analyses were performed using comparison tests and correlation analysis.

Results: a significant decrease in hemoglobin levels (9.37 ± 0.50 vs. 14.18 ± 0.64 g/dL) and hematocrit (28.63 ± 1.40 vs. 40.69 ± 1.91), compared to a significant increase in urea (124.38 ± 17.39 vs. 29.64 ± 2.25 mg/dL) and creatinine (8.16 ± 0.63 vs. 0.75 ± 0.03 mg/dL) ($p < 0.001$). At the molecular level, significant increases in the gene expression of CASP8 (46.09 ± 6.70 folds) and CASP9 (13.59 ± 1.51 folds) were observed, along with elevated levels of PDCD1 (25.66 ± 4.19), CTLA4 (34.69 ± 8.43), and TGFB3 (15.29 ± 2.83).

Conclusion: These findings suggest a complex regulatory network involving apoptosis activation, immunosuppression, and fibrosis in patients with renal failure. These results highlight an integrated molecular axis that may represent a promising target for future therapeutic interventions and provide a foundation for a deeper understanding of the pathogenic mechanisms underlying disease progression.

Introduction

Chronic kidney disease (CKD) represents one of the most significant contemporary global health challenges due to its high prevalence and profound impact on morbidity and mortality rates. Recent estimates indicate that more than 850 million people worldwide suffer from kidney disease at various stages, with a particularly high disease burden in low- and middle-income countries [1]. CKD is characterized by a progressive and irreversible decline in kidney function, leading in its final stages to end-stage renal disease (ESRD), which requires costly treatment interventions such as dialysis or kidney transplantation [2]. At the molecular level, disruption of the balance between cell survival and death is a key driver of CKD progression. Programmed cell death (apoptosis) plays a pivotal role in renal cell loss, with its intrinsic and extrinsic pathways activated by caspases, particularly CASP8 and CASP9. Recent studies have shown that the over activation of these molecules is directly associated with renal epithelial cell damage and increased renal injury severity, as well as promoting local inflammation [3, 4]. Furthermore, continued activation of apoptosis pathways contributes to the loss of renal tissue integrity and accelerates disease progression. Furthermore, CKD patients are characterized by profound immune system dysregulation, leading to a state of chronic immunosuppression known as T-cell exhaustion. Immune checkpoint molecules such as PD-1 (PDCD1) and CTLA-4 are among the most important

negative regulators of the immune response, and their elevated expression in CKD patients has been shown to be associated with impaired immune response and an increased risk of infection and chronic inflammation [5]. Recent evidence suggests that these molecules not only regulate immunity but also contribute to the remodeling of the tissue environment and promote pathological processes such as fibrosis [6]. Renal fibrosis is the common end stage of most chronic kidney diseases, characterized by the accumulation of extracellular matrix and loss of kidney function. Transforming growth factor-beta (TGF- β), particularly TGF- β 3, is a key molecular driver of this process, inducing epithelial-stromal transformation (EMT) and fibroblast activation, leading to collagen deposition and renal tissue destruction [7]. Activation of the TGF- β pathway is also linked to Smad signaling, which controls gene expression associated with fibrosis and kidney dysfunction [8]. Recent evidence points to a complex dynamic interplay between cell death pathways, immune checkpoints, and fibrosis. Studies have shown that caspase activation can enhance PD-1 expression, creating a feedback loop that exacerbates immunosuppression and increases tissue damage [9]. Chronic inflammation and activation of pathways such as the NLRP3 inflammasome also link apoptosis and fibrosis by stimulating the release of fibrotic cytokines such as TGF- β [10]. This multi-layered interplay reflects the complex nature of CKD progression and underscores the importance of studying these pathways in an integrated rather than isolated manner.

This study aims to analyze the gene expression patterns of genes associated with cell death pathways (CASP8 and CASP9), immune checkpoints (PDCD1 and CTLA4), and fibrosis (TGFB3) in kidney failure patients, while assessing the correlational relationships between them and clinical and biochemical indicators, in order to understand the molecular interaction between these pathways and their role in disease progression.

Material and Methods

Subject collection

The study recruited a total of 60 participants between March 2024 and February 2025. medical city dialysis center 30 patients diagnosed with Renal failure, while 30 person served as healthy controls.

Inclusion Criteria

For Patients with Renal Failure (Case Group) Age between 22 and 57 years. Diagnosed with end-stage renal disease (ESRD) or chronic renal failure, confirmed by specialized nephrologists based on clinical and laboratory findings. Undergoing regular hemodialysis at the Medical City Dialysis Center. Blood samples were collected prior to dialysis sessions and from patients scheduled for their first kidney transplant from a living donor, one week before transplantation. Stable clinical condition for at least 1 month prior to enrollment. Willing and able to provide informed consent.

For Healthy Controls: Age between 25 and 55 years. No history of kidney disease or any chronic systemic illness. Normal renal function tests (serum creatinine and urea within normal limits). Not currently taking any immunosuppressive or anti-inflammatory medications. Willing and able to provide informed consent.

Exclusion Criteria (Both Groups)

History of autoimmune disorders (e.g., lupus, rheumatoid arthritis). Recent infections or active inflammatory conditions (within the past 4 weeks). Diagnosed malignancy or receiving chemotherapy or radiotherapy. History of organ transplantation. Pregnant or breastfeeding women. Inability or unwillingness to provide informed consent. Any mental or cognitive disorder that could interfere with participation in the study.

Blood Collection and Processing

Approximately 5–7 mL of venous blood was collected from each participant using sterile disposable syringes under aseptic conditions. For renal failure patients, samples were collected prior to dialysis sessions and from patients scheduled for their first kidney transplant one week before surgery. Fasting blood was divided into:

- • EDTA tubes for hematological tests (Hemoglobin and Hematocrit).
- • Plain tubes for serum separation. Serum was obtained by centrifugation at 3000 rpm for 10 minutes and stored at – 20°C until biochemical analysis.
- • Two hundred fifty microliters (0.25 mL) of whole blood were combined from each participant (patients and controls) with 0.5 mL of TRIzol reagent within a 1.5 mL Eppendorf tube for molecular study. This mixture was stored at -20°C for further molecular analysis.

Hematological Tests

Hemoglobin (Hb) and Hematocrit (HCT) were measured using the Mindray Hematology Analyzer based on automated electrical impedance and photometric analysis. These parameters help assess anemia in renal failure patients. Normal ranges: Hb (13.5–17.5 g/dL males, 12.0–15.5 g/dL females), HCT (38.8–50.0% males, 34.9–44.5% females).

Biochemical Tests

Biochemical analyses including blood urea, serum creatinine, and serum glucose were conducted using the Cobas C311 Autoanalyzer (Roche Diagnostics). Blood urea with a normal range of 16.60–48.50 mg/dL. Serum creatinine with a reference range of 0.50–0.90 mg/dL. Serum glucose normal fasting range was 90–120 mg/dL.

Molecular Study

The primers of Real-time polymerase chain reaction (RT-PCR)

Macrogen Company provided lyophilized primers BECN1 and PDCD1 genes and GAPDH (housekeeping gene used as control). The primers were reconstituted in nuclease-free water to create a 100 pmol/μL stock solution. Dilute 10 μL of primer stock solution with 90 μL of nuclease-free water to reach a final concentration of 10 pmol/μL for working solutions. All dilutions were nuclease-free to reduce primer degradation. For later usage, stock solutions were stored at -20°C.

The primer sequences used for quantitative re-al-time PCR (qRT-PCR) analysis are summarized as follows. For CASP8, the forward primer se-quence was 5'-CTTGGATGCAGGGGCTTTGACC-3' and the reverse primer sequence was 5'-GTTCACTTCAGTCAGGATGG-3', generating a 123 bp amplicon at an annealing temperature of 60°C [11]. For CASP9, the forward primer sequence was 5'-ACAGGGTCTGCTCTTTCT-3' and the reverse primer sequence was 5'-GCATTCATCTGTCCCTCTTC-3', producing a 132 bp PCR product at an annealing temperature of 60°C [12]. For PDCD1, the forward primer se-quence was 5'-TAGAGAAGTTTCAGGGAAGG-3' and the reverse primer sequence was 5'-ATGTGTAAAGGTGGAGGG-3', generating a 77 bp amplicon at 60°C [13]. For CTLA4, the forward primer sequence was 5'-CTTCAGTCACCTGGCTGTCA-3' and the re-verse primer sequence was 5'-CTCAGCTGAACCTGGCTACC-3', producing a 219 bp PCR product at an annealing temperature of 60°C [14]. For TGFB3, the forward primer se-quence was 5'-CTGGATTGTGGTTCCATGCA-3' and the reverse primer sequence was 5'-TCCCCGAATGCCTCACAT-3', generating a 122 bp amplicon at 60°C [14]. GAPDH was used as the housekeeping gene, with a forward primer sequence of 5'-TGATGACATCAAGAAGGTGGTGAAG-3' and a reverse primer sequence of 5'-TCCTTGGAGGCCATGTAGGCCAT-3'. The ex-pected product size was 226 bp, and the annealing temperature was 65°C [15].

Extraction and determination of RNA

Total RNA, including small RNA fractions (miRNAs), was extracted from peripheral blood using TRIzol™ LS reagent (Thermo Fisher Scientific, USA) with slight modifications to optimize miRNA recovery. Procedures were performed using RNase-free consumables and, where applicable, under cold conditions. About 250 μL of chloroform was incubated and shaken vigorously then separated into organic and aqueous phases (12,000× g, 15 min, 4°C). RNA was precipitated with isopropanol, washed with 75% ethanol, air-dried, and dissolved in nuclease-free water. RNA purity and concentration were evaluated using NanoDrop™ 2000 (acceptable A260/A280 = 1.8–2.1), and integrity was assessed on 1.5% agarose gel electrophoresis. RNA was stored at – 80°C until analysis.

Reverse transcription (cDNA synthesis)

For mRNA targets, cDNA was synthesized using the GoScript™ Reverse Transcription System (Promega, USA) according to the manufacturer's instructions.

Quantitative real-time PCR (qPCR)

Quantitative real-time PCR was performed using SYBR™ Green Master Mix (Applied Biosystems, USA) on a qTOWER³ G instrument (Analytik Jena, Germany). Each 20 µL reaction contained 10 µL 2× SYBR mix, 1 µL forward primer (10 µM), 1 µL reverse primer (10 µM), 2 µL cDNA, and 6 µL nuclease-free water. Cycling conditions were: 95°C for 10 min; 40 cycles of 95°C for 15 s and 60°C for 30 s; followed by melt-curve analysis (65–95°C, 0.5°C increments). All reactions were run in duplicate with non-template controls.

Normalization and relative quantification

Expression data were normalized using GAPDH for mRNA targets and relative expression was calculated using the $2^{-\Delta\Delta C_t}$ method [16].

Gene Expression Quantification

Relative gene expression levels were calculated using the Livak method ($2^{-\Delta\Delta C_t}$):

- ΔC_t : Calculated as the difference between the C_t value of the target gene and the housekeeping gene (GAPDH).
- $\Delta\Delta C_t$: Calculated as the difference between the ΔC_t of the sample (treated or experimental) and the ΔC_t of the control group.

The housekeeping gene GAPDH was selected as an internal control due to its stable expression across the studied samples. This method allowed accurate quantification of the relative expression levels of CASP8, CASP9, PDCD1, CTLA4 and TGFB3, ensuring reproducibility and reliability of the results.

Statistical analysis

The statistical studies were done in GraphPad Prism v10.0 and SPSS v26.0. Data normality was checked before choosing statistical tests. Using normally distributed data, independent t-test and One-way analysis of variance (ANOVA) were used as appropriate were used to compare renal failure patients with control. Then, post-hoc testing with multiple comparison corrections was carried out to identify group differences. Person correlation coefficient test was used to find out the correlation between the studied parameters. All data were presented as mean $\pm$ standard error (SE), and $p \leq 0.05$, was considered as significant difference.

Results

Clinical Parameters

The comparison between renal failure patients and health controls, based on hematological and biochemical profiles demonstrated significant differences across all measureable parameters (Fig. 1; panel A-E). Hemoglobin levels in renal patients were significantly decreased (9.37 ± 0.50 ; $p < 0.001$) g/dL, compared to controls (14.18 ± 0.64) g/dL. Similarly, the hematocrit (HCT) values. A marked significant reduced HCT demonstrated in renal failure patients (28.63 ± 1.40 ; $p < 0.001$) than controls (40.69 ± 1.91). In contrast, blood urea and serum creatinine as renal function markers, significantly elevated in renal failure patients group compared to controls ($p < 0.001$). Blood urea levels were substantially higher (124.38 ± 17.39 mg/dL; $p < 0.001$) in renal failure patients vice versa control (29.64 ± 2.25) mg/dL. In the same line, serum creatinine levels recorded pronounced elevation (8.16 ± 0.63 mg/dL; $p < 0.001$) versus healthy controls (0.75 ± 0.03) mg/dL. In addition, glucose levels in renal failure patients were significantly higher than controls (154.201 ± 21.66 vs. 99.45 ± 1.72 mg/dL; $p = 0.03$).

Relative gene expression analysis

Apoptotic gene expression

The levels of apoptosis-related genes expression revealed significant upregulation in renal failure patients compared to healthy controls as presented in (Fig. 2; panel A and B). The expression of CASP8 markedly increased in renal failure patients (38.80 ± 3.26 fold) compared with controls (1.92 ± 0.61 fold), corresponding to an overall fold change of 20.25 ($p < 0.0001$). Similarly, CASP9 expression were significantly elevated in renal failure patients (19.71 ± 3.94 fold) relative to healthy controls (1.45 ± 0.44 fold), corresponding to an overall fold change of 13.59 ($p < 0.0001$).

Immune Checkpoint Gene Expression

Immune checkpoint-related genes analysis also demonstrated significant upregulation in renal failure patients (Fig. 2; panel C and D). The relative PDCD1 expression was significantly higher in renal failure patients (35.40 ± 7.18 fold) compared with controls (1.90 ± 0.60 fold), corresponding to an overall fold change of 18.63 ($p < 0.0001$). Likewise, the relative CTLA4 expression showed a pronounced increase in the folding of renal failure patients (28.79 ± 7.65 fold) relative to healthy controls (2.21 ± 0.70 fold), corresponding to an overall fold change of 13.03 ($p < 0.0001$).

Fibrosis-Associated Gene Expression

Fibrosis-related gene TGFB3 relative expression significantly increased in renal failure patients (Fig. 2; panel E). The renal failure patients exhibited higher TGFB3 expression levels (25.15 ± 5.13 fold) compared with controls (2.11 ± 0.89 fold), corresponding to an overall fold change of 11.92 ($p < 0.0001$).

Correlation analysis

Pearson correlation analysis revealed that, significant associations among apoptosis-related, immune checkpoint, and fibrosis-related genes in renal failure patients (Fig. 3). Strong positive correlations between CASP8 and CASP9 ($r = 0.711$, $P = 0.001$), PDCD1 ($r = 0.597$, $P = 0.005$), TGFB3 ($r = 0.684$, $P =$

0.001), and CTLA4 ($r = 0.465$, $P = 0.039$). Additionally, strongly correlated analysis between CASP9 with CTLA4 ($r = 0.792$, $P = 0.001$), PDCD1 ($r = 0.485$, $P = 0.030$), and TGFB3 ($r = 0.573$, $P = 0.008$). CTLA4 expression was significant correlated with PDCD1 ($r = 0.373$, $P = 0.003$) and TGFB3 ($r = 0.510$, $P = 0.021$), while PDCD1 also correlated with TGFB3 ($r = 0.607$, $P = 0.005$). All studied genes exhibited negative correlations with hematological profile, specifically HGb. and HCT. Notably, CASP9 showed strong negative correlations with HGb. and HCT ($r = -0.813$, $P = 0.001$, $r = -0.724$, $P = 0.001$) respectively. Furthermore, gene expression levels correlated to RFT. Strong correlation recorded between CASP8 and serum creatinine ($r = 0.849$, $P = 0.001$), while TGFB3 was correlated to blood urea levels ($r = 0.736$, $P = 0.001$). Moderate correlations between CASP9, CTLA4, and glucose levels ($r = 0.579$, $P = 0.007$; $r = 0.518$, $P = 0.019$) respectively, while non-significant correlation recorded with CASP8, PDCD1 and TGFB3.

Discussion

The results of this study demonstrate clear molecular imbalances in apoptosis pathways, immune checkpoints, and fibrosis in patients with renal failure, reflecting the complex interplay between chronic inflammation, oxidative stress, and impaired immune response in disease progression.

The significantly elevated gene expression of both CASP8 and CASP9 and strong correlation between them, underscores the central role of programmed cell death in the development of renal failure, as these genes are key switches for both the extrinsic and intrinsic pathways of apoptosis. CASP8 is primarily activated through death receptor signaling, including TNF- α and Fas/FasL pathways, whereas CASP9 is activated through mitochondrial dysfunction and oxidative stress [17, 18]. Chronic kidney disease is characterized by persistent inflammation, uremic toxin accumulation, and excessive reactive oxygen species (ROS) generation, all of which promote activation of these apoptotic cascades [19]. Renal and hematopoietic cells are subjected to continuous stress, leading to the induction of apoptosis [20]. These findings reflect a multidimensional molecular disorder in patients with renal failure, characterized by the simultaneous activation of apoptosis pathways, immunosuppression, and fibrosis. Recent studies have shown that regulated cell death patterns play a crucial role in kidney diseases, including chronic kidney disease, by promoting renal cell loss and increasing tissue inflammation [21]. Other research has indicated that caspase activation is associated with worsening kidney damage and a poor prognosis, especially when combined with oxidative stress and metabolic homeostasis [22]. Recent studies have shown that dysregulated cell death is a key mechanism for nephron loss and chronic kidney disease progression [4].

The strong positive correlation between CASP8/9 and creatinine and urea levels in this study reflects the close relationship between cell death and deteriorating kidney function. Recent studies have shown that increased caspase activity is associated with poor prognostic indicators in CKD and contributes to the loss of renal tissue integrity through the destruction of epithelial cells [21]. In addition, the inverse relationship with hemoglobin and hematocrit may indicate a role for apoptosis in the exacerbation of anemia associated with renal failure, which has recently been documented in the context of chronic inflammation and erythropoietin deficiency. On the other hand, the study showed a marked increase in

the gene expression of both PDCD1 (PD-1) and CTLA4, indicating a state of chronic immunosuppression or T-cell exhaustion. Recent studies support this concept, showing that CKD patients suffer from immune system dysfunction characterized by increased expression of PD-1 and PD-L1, leading to suppression of the immune response and an increased risk of infection [23]. Furthermore, elevated PD-1 is not only an immunological marker but is also associated with the progression of renal fibrosis, having been found to be an independent risk factor for fibrosis progression in patients with nephropathy [24].

CTLA4 is a key early regulator of T cell activation, and recent studies have shown that its increase is associated with immunosuppression and altered inflammatory environments in the kidneys, contributing to disease progression. The positive correlation between PD-1 and CTLA4 in this study reflects an integrated immunosuppression axis, consistent with recent models suggesting that these pathways work synergistically to regulate the immune response [25, 26]. Most importantly, the correlation results demonstrated a strong relationship between apoptosis genes and immune checkpoints, suggesting a functional overlap between cell death and immune regulation. Recent studies have shown that chronic inflammatory signaling can link these pathways, with caspase activation promoting PD-1 expression, creating a feedback loop that perpetuates tissue damage. Furthermore, some studies suggest that PD-1 pathways may directly influence cell survival and apoptosis regulation, further reinforcing this correlation [23, 27]. CTLA4 is a key early regulator of T cell activation, and recent studies have shown that its increase is associated with immunosuppression and altered inflammatory environments in the kidneys, contributing to disease progression. The positive correlation between PD-1 and CTLA4 in this study reflects an integrated immunosuppression axis, consistent with recent models suggesting that these pathways work synergistically to regulate the immune response [28, 29].

Regarding the TGFB3 gene, the results showed a significant increase, consistent with its known role in renal fibrosis. TGF- β is one of the most important fibrosis-promoting cytokines, as it promotes extracellular matrix deposition and induces epithelial-stromal transformation (EMT), leading to loss of kidney structure and function [30]. Furthermore, activation of the TGF- β pathway is associated with the activation of Smad proteins, which regulate gene expression related to fibrosis [31]. Recent studies indicate that renal fibrosis is the end result of most chronic kidney diseases, characterized by collagen buildup and fibroblast proliferation, which leads to the destruction of the kidney's tissue structure [32]. Immune cells, particularly macrophages, also play a significant role in this context by secreting fibrotic factors such as TGF- β , which promotes disease progression [21].

The positive correlation between TGFB3 and CASP8, PDCD1, and CTLA4 in this study reflects a complex regulatory network linking fibrosis, apoptosis, and immunity. Recent studies have shown that inflammatory pathways such as the NLRP3 inflammasome can connect these processes, with their activation inducing both apoptosis and fibrosis [33]. Pyroptosis, another form of cell death, also contributes to increased TGF- β secretion and thus accelerates fibrosis [34]. Clinically, these findings suggest that chronic kidney disease is a complex condition involving an ongoing interplay between inflammation, immunity, and cell death. Recent studies have shown that targeting these pathways may offer promising therapeutic opportunities, such as inhibiting cell death pathways or modulating immune

checkpoint activity. However, these strategies remain in the research phase due to the complexity of the immune homeostasis in the kidney and the risk of side effects. Despite the importance of these findings, some limitations must be considered, such as the small sample size, which may affect statistical power. Furthermore, the study relied solely on gene expression analysis without protein analysis, which may not reflect the full functional activity. Therefore, recent research recommends using multiple techniques, such as proteomics and single-cell sequencing, for a deeper understanding of these pathways.

Conclusion

In this study, we observed that the genes associated with apoptosis (CASP8 and CASP9), immune checkpoint molecules (PDCD1 and CTLA4) and the fibrosis-related gene TGFB3 were significantly upregulated in the patients with renal failure compared with the healthy controls. The observed positive correlations of these genes and their association with markers of renal dysfunction suggest a highly interconnected molecular network linking apoptosis, immune dysregulation, and fibrotic remodelling during disease progression. These findings suggest that renal failure is due to a complex pathogenic axis rather than isolated molecular events. Hence, CASP8, CASP9, PDCD1, CTLA4 and TGFB3 could be potential biomarkers for monitoring the disease and potential therapeutic targets.

Abbreviations

Chronic Kidney Disease (CKD); End-Stage Renal Disease (ESRD); Reverse Transcription Quantitative Polymerase Chain Reaction (RT-Qpcr); Quantitative Polymerase Chain Reaction (qPCR); Ribonucleic Acid (RNA); Complementary DNA (cDNA); Cycle Threshold (Ct); Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH); Caspase-8 (CASP8); Caspase-9 (CASP8); Programmed Cell Death Protein 1 Gene (PDCD1); Programmed Cell Death Protein 1 (PD-1); Cytotoxic T-Lymphocyte-Associated Protein 4 (CTLA4); Transforming Growth Factor Beta 3 (TGFB3); Transforming Growth Factor Beta (TGF- β); Epithelial-to-Mesenchymal Transition (EMT); Reactive Oxygen Species (ROS); Tumor Necrosis Factor Alpha (TNF- α); Fas Receptor/Fas Ligand (Fas/FasL); NOD-, LRR-, and Pyrin Domain-Containing Protein 3 (NLRP3); Hemoglobin (Hb); Hematocrit (HCT); Renal Function Test (RFT); Ethylenediaminetetraacetic Acid (EDTA); Thiocyanate–Phenol–Chloroform RNA Isolation Reagent (TRIzol).

Declarations

Acknowledgements

The authors would like to thank Mustansiriyah University and University of Baghdad providing laboratory facilities and technical support during this study.

Funding

This work received no funding.

Competing interests

The authors declare that they have no competing interests.

Ethics approval

The study protocol was approved by the local Institutional Review Board/Ethics Committee in the Department of Pharmacology and Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq (Approval No. 61 on 5/6/2025). All procedures were conducted in accordance with the Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from all participants before sample collection.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Code availability

Not applicable.

Authors' contributions

Conceptualization, all authors; methodology, Abdullahl, M.R.; software, Al-Zaidi, O.S.S.H; validation, all authors; formal analysis, all authors; investigation, Abdullahl, M.R.; resources, all authors; data curation, all authors; writing-original draft preparation, all authors; writing—review and editing, all authors; visualization, all authors; funding acquisition, all authors. All authors have read and agreed to the published version of the manuscript.

References

1. Romagnani P, Agarwal R, Chan JCN, et al. Chronic kidney disease. Nat Rev Dis Primers. 2025;11:8. <https://doi.org/10.1038/s41572-024-00589-9>.
2. Miguel V, Shaw IW, Kramann R. Metabolism at the crossroads of inflammation and fibrosis in chronic kidney disease. Nat Rev Nephrol. 2025;21(1):39–56. <https://doi.org/10.1038/s41581-024-00889-z>.

3. Deng L, Guo S, Liu Y, Zhou Y, Liu Y, Zheng X, Yu X, Shuai P. Global, regional, and national burden of chronic kidney disease and its underlying etiologies from 1990 to 2021: a systematic analysis for the Global Burden of Disease Study 2021. *BMC Public Health*. 2025;25(1):636. <https://doi.org/10.1186/s12889-025-21851-z>.
4. Sanz AB, Sanchez-Niño MD, Ramos AM, Ortiz A. Regulated cell death pathways in kidney disease. *Nat Rev Nephrol*. 2023;19(5):281–99. <https://doi.org/10.1038/s41581-023-00694-0>.
5. Kurts C, von Vietinghoff S, Krebs CF, Panzer U. Kidney immunology from pathophysiology to clinical translation. *Nat Rev Immunol*. 2025;25(6):460–76. <https://doi.org/10.1038/s41577-025-01131-y>.
6. Bermejo S, Bolufer M, Riveiro-Barciela M, Soler MJ. Immunotherapy and the Spectrum of Kidney Disease: Should We Individualize the Treatment? *Front Med*. 2022;9:906565. <https://doi.org/10.3389/fmed.2022.906565>.
7. Meng XM, Nikolic-Paterson DJ, Lan HY. TGF- β : the master regulator of fibrosis. *Nat Rev Nephrol*. 2016;12(6):325–38. <https://doi.org/10.1038/nrneph.2016.48>.
8. Rajanna S, Kedlaya PG, Satish R, Muthugopalakrishnan G. WCN23-0948 Changing trends of pregnancy related acute kidney injury: A single-centre prospective observational study from southern India. *Kidney Int Rep*. 2023;8(3):S19–20. <https://doi.org/10.1016/j.ekir.2023.02.1036>.
9. Vandikas MS, Landin-Wilhelmsen K, Gillstedt M, Osmancevic A. Vitamin D-Binding Protein and the Free Hormone Hypothesis for Vitamin D in Bio-Naïve Patients with Psoriasis. *Int J Mol Sci*. 2022;23(3):1302. <https://doi.org/10.3390/ijms23031302>.
10. Luo Y, Long M, Wu X, Zeng L. Targeting the NLRP3 inflammasome in kidney disease: molecular mechanisms, pathogenic roles, and emerging small-molecule therapeutics. *Front Immunol*. 2025;16:1703560. <https://doi.org/10.3389/fimmu.2025.1703560>.
11. Liedtke C, Groger N, Manns MP, Trautwein C. The human caspase-8 promoter sustains basal activity through SP1 and ETS-like transcription factors and can be up-regulated by a p53-dependent mechanism. *J Biol Chem*. 2003;278(30):27593–604. <https://doi.org/10.1074/jbc.M304077200>.
12. Dirican E, Özcan H, Uzunçakmak K, S., Takım U. Evaluation Expression of the Caspase-3 and Caspase-9 Apoptotic Genes in Schizophrenia Patients. *Clin Psychopharmacol neuroscience: official Sci J Korean Coll Neuropsychopharmacol*. 2023;21(1):171–8. <https://doi.org/10.9758/cpn.2023.21.1.171>.
13. Abdullah MR, Alabassi HM. The Synergistic Effect of Autophagy and Apoptosis in Iraqi Women with Polycystic Ovary Syndrome. *J Reprod infertility*. 2025;26(1):36–49. <https://doi.org/10.18502/jri.v26i1.18780>.
14. Abdullah MR, Alabassi HM. The potential influence of immune modulatory molecules (TGF- β III and CTLA-4) in the pathogenicity of PCOS. *Karbala Int J Mod ScienceKarbala Int J Mod Sci*. 2024;10(3):363–75. <https://doi.org/10.33640/2405-609X.3372>.
15. Kwon N, Lee KE, Singh M, Kang SG. Suitable primers for GAPDH reference gene amplification in quantitative RT-PCR analysis of human gene expression. *Gene Rep*. 2021;24:101272. <https://doi.org/10.1016/j.genrep.2021.101272>.

16. Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 – $\Delta\Delta CT$ method. *methods*, 25(4), pp.402–408.
<https://doi.org/10.1006/meth.2001.1262>
17. Chen H, Lin Y, Chen J, Luo X, Kan Y, He Y, Zhu R, Jin J, Li D, Wang Y, Han Z. Targeting caspase-8: a new strategy for combating hepatocellular carcinoma. *Front Immunol*. 2024;15:1501659.
<https://doi.org/10.3389/fimmu.2024.1501659>.
18. Liang Y, Han W, Xu C, Wang J, Zhang J, An J, Liu W, Liu J, Zhang Z, Shi J, Zhang K. Cascade-Responsive Oxidative Stress Amplifiers Simultaneously Destroy Lysosomes and Co-Deliver CRISPR/Cas9 to Enhance Oxidative Damage in Tumor. *Adv Funct Mater*. 2023;33(32):2301256.
<https://doi.org/10.1002/adfm.202301256>.
19. Frąk W, Dąbek B, Balcerczyk-Lis M, Motor J, Radzioch E, Młynarska E, Rysz J, Franczyk B. Role of Uremic Toxins, Oxidative Stress, and Renal Fibrosis in Chronic Kidney Disease. *Antioxid (Basel Switzerland)*. 2024;13(6):687. <https://doi.org/10.3390/antiox13060687>.
20. Jing Z, Chen Q, Yan C, Zhang C, Xu Z, Huang X, Wu J, Li Y, Yang S. Effects of Chronic Heat Stress on Kidney Damage, Apoptosis, Inflammation, and Heat Shock Proteins of Siberian Sturgeon (*Acipenser baerii*). *Animals: open access J MDPI*. 2023;13(23):3733. <https://doi.org/10.3390/ani13233733>.
21. Li Y, Zhang J, Qiu X, Zhang Y, Wu J, Bi Q, Sun Z, Wang W. Diverse regulated cell death patterns and immune traits in kidney allograft with fibrosis: a prediction of renal allograft failure based on machine learning, single-nucleus RNA sequencing and molecular docking. *Ren Fail*. 2024;46(2):2435487. <https://doi.org/10.1080/0886022X.2024.2435487>.
22. Cao Y, Hu B, Fan Y, Wang W, Chi M, Nasser MI, Ma K, Liu C. The effects of apoptosis inhibitor of macrophage in kidney diseases. *Eur J Med Res*. 2024;29(1):21. <https://doi.org/10.1186/s40001-023-01597-3>.
23. Chen R, Lin Q, Tang H, Dai X, Jiang L, Cui N, Li X. PD-1 immunology in the kidneys: a growing relationship. *Front Immunol*. 2024;15:1458209. <https://doi.org/10.3389/fimmu.2024.1458209>.
24. Zhang Y, Mi X, Zhang Y, Li J, Qin Y, He P, Zhao Y, Su B, He L. Immune checkpoint activity exacerbate renal interstitial fibrosis progression by enhancing PD-L1 expression in renal tubular epithelial cells. *Translational research: J Lab Clin Med*. 2024;271:52–67. <https://doi.org/10.1016/j.trsl.2024.05.004>.
25. Lin X, Kang K, Chen P, Zeng Z, Li G, Xiong W, Yi M, Xiang B. Regulatory mechanisms of PD-1/PD-L1 in cancers. *Mol Cancer*. 2024;23(1):108. <https://doi.org/10.1186/s12943-024-02023-w>.
26. Alturki NA. Review of the Immune Checkpoint Inhibitors in the Context of Cancer Treatment. *J Clin Med*. 2023;12(13):4301. <https://doi.org/10.3390/jcm12134301>.
27. Qu Y, Lin Z, Yang Z, Lin H, Huang X, Gu L. Machine learning models for prognosis prediction in endodontic microsurgery. *J Dent*. 2022;118:103947. <https://doi.org/10.1016/j.jdent.2022.103947>.
28. Borgeaud M, Sandoval J, Obeid M, Banna G, Michielin O, Addeo A, Friedlaender A. Novel targets for immune-checkpoint inhibition in cancer. *Cancer Treat Rev*. 2023;120:102614.
<https://doi.org/10.1016/j.ctrv.2023.102614>.

29. Tan J, Xue Q, Hu X, Yang J. Inhibitor of PD-1/PD-L1: a new approach may be beneficial for the treatment of idiopathic pulmonary fibrosis. *J translational Med.* 2024;22(1):95. <https://doi.org/10.1186/s12967-024-04884-7>.
30. Huang R, Fu P, Ma L. Kidney fibrosis: from mechanisms to therapeutic medicines. *Signal Transduct Target therapy.* 2023;8(1):129. <https://doi.org/10.1038/s41392-023-01379-7>.
31. Alruhaimi RS, Hassanein EHM, Alnasser SM, Alzoghaibi MA, El-Ghafar A, Mohammad OAM, Elbagory MK, I., Mahmoud AM. The sesquiterpene alcohol farnesol mitigates cadmium hepatotoxicity by attenuating oxidative stress and NF-kappaB/NLRP3 inflammasome axis and upregulating PPARgamma in rats. *EXCLI J.* 2024;23:1356–74. <https://doi.org/10.17179/excli2024-7488>.
32. Liu Y, Lei H, Zhang W, Xing Q, Liu R, Wu S, Liu Z, Yan Q, Li W, Liu X, Hu Y. Pyroptosis in renal inflammation and fibrosis: current knowledge and clinical significance. *Cell Death Dis.* 2023;14(7):472. <https://doi.org/10.1038/s41419-023-06005-6>.
33. Ji Y, Hua H, Jia Z, Zhang A, Ding G. Therapy Targeted to the NLRP3 Inflammasome in Chronic Kidney Disease. *Kidney diseases. (Basel Switzerland).* 2024;10(5):369–83. <https://doi.org/10.1159/000539496>.
34. Tian Y, Mushuo Q, Fan H, Su H, Li J, Zhang Q. Targeting pyroptosis in renal fibrosis: From molecular mechanisms to therapeutic horizons (Review). *Int J Mol Med.* 2026;57(5):133. <https://doi.org/10.3892/ijmm.2026.5804>.

Figures

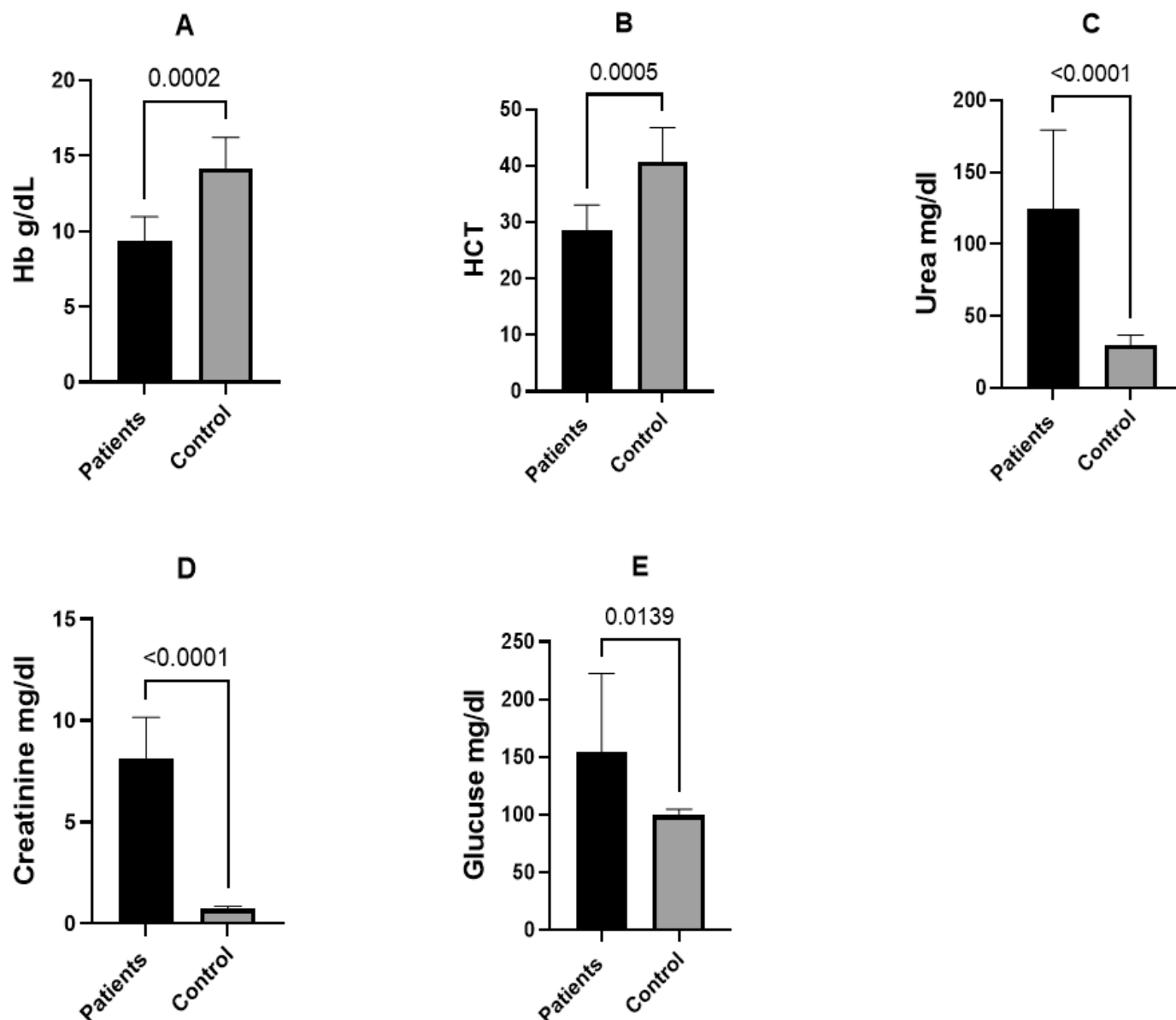


Figure 1

Hematological and biochemical parameters Comparison between RFPs and healthy controls. Bar graphs data represent (mean $\pm$ SE.) values of hematological parameters and biochemical parameters, including panel (A): hemoglobin, panel (B): hematocrit, panel (C): blood urea, panel (D): serum creatinine, and panel (E): glucose levels. Renal failure patients exhibited significantly ($p < 0.001$) lower HGb and HCT levels, alongside significantly elevated (urea, and creatinine; $p < 0.001$), and glucose levels ($p = 0.03$) relative to controls. *** $p < 0.001$. RFPs= Renal failure patients.

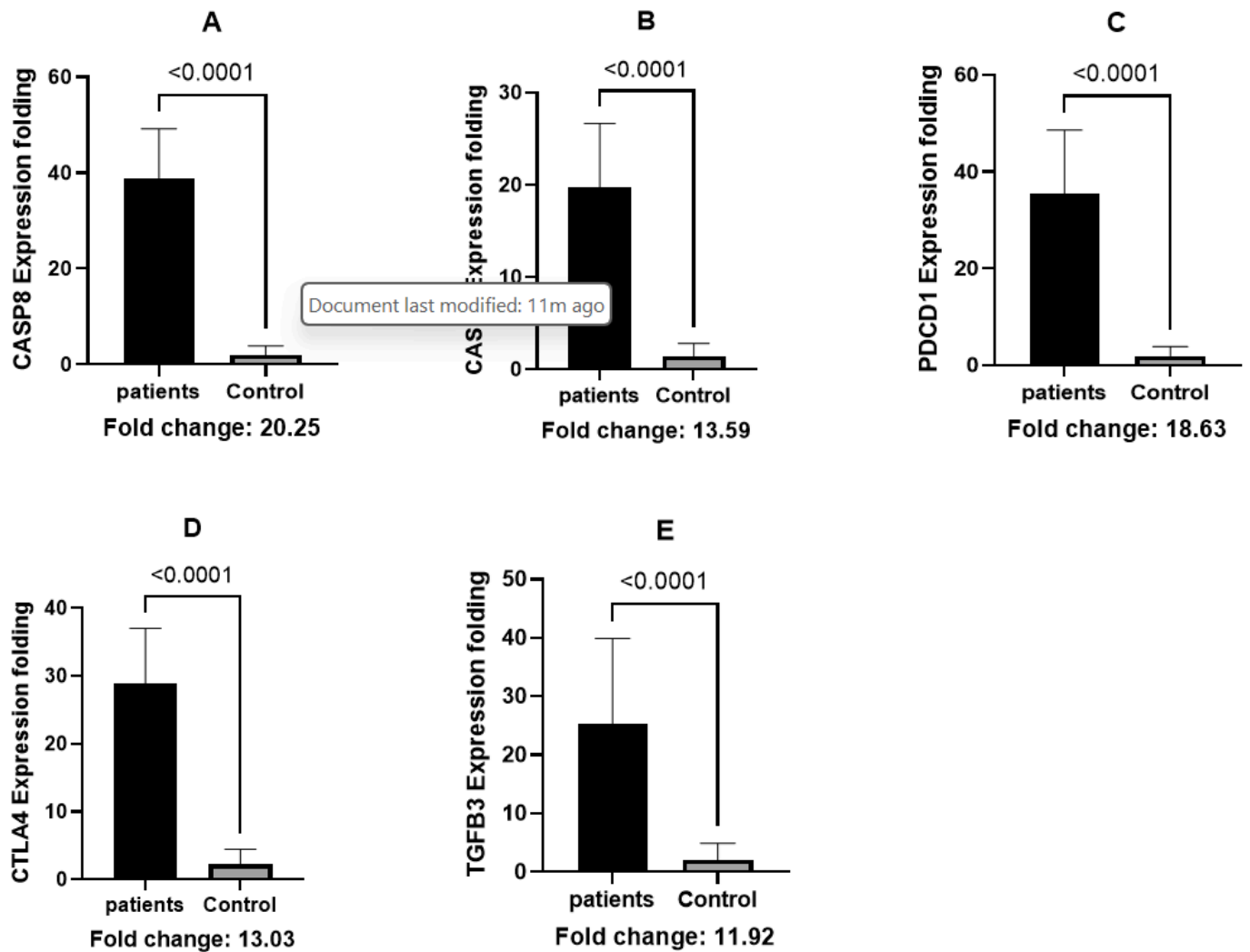


Figure 2

Upregulation of apoptosis, immune checkpoint, and fibrosis-associated gene expression in RFTs. Data of bar graph represent (mean $\pm$ SE.) of renal failure patients related gene expression compared to healthy controls. Panels (A): CASP8, (B): CASP9, (C): PDCD1, (D): CTLA4, and (E): TGFB3 represent target genes. All genes exhibited significant relative upregulation ($p < 0.05$) in renal failure patients to health controls. *** $p < 0.001$. RFPs= Renal failure patients.

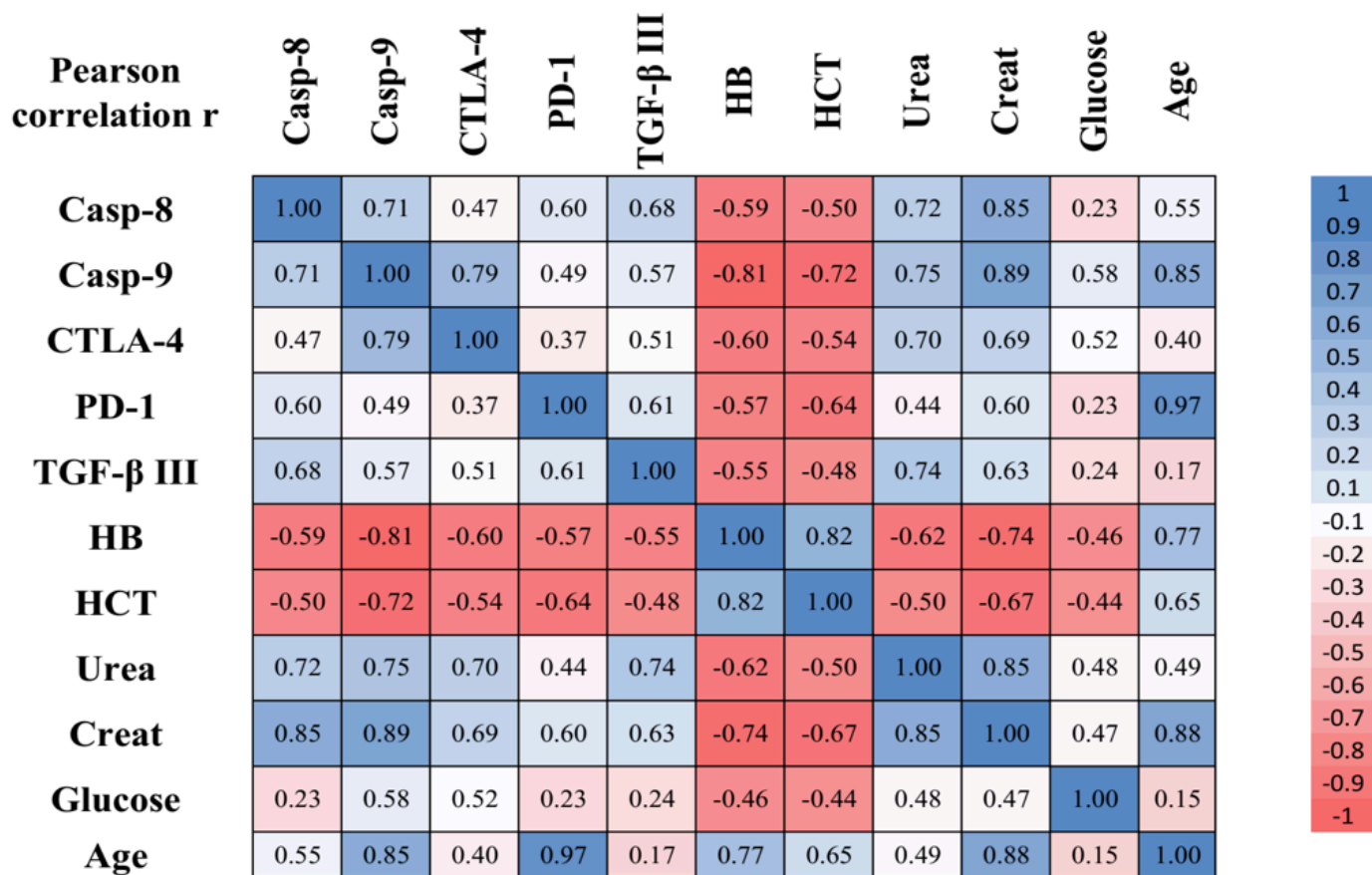


Figure 3

Correlation matrix heatmap of various biomarkers in the renal failure patients (blue color indicates direct correlation, while red color indicates inverse correlation; value represents correlation coefficient, and value above 0.25 indicates significant correlation).