Original Article

Homocysteine Level and Some Biochemical Variables in Kidney Patients, Isolation and Partial Purification of Methionine Synthase Enzyme, Preparation of a Compound and its Effect on the Isolated Enzyme

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ABSTRACT

Objective: To evaluate serum homocysteine, malondialdehyde, glutathione peroxidase, and tumor necrosis factor-alpha levels in hemodialysis and non-dialysis chronic kidney disease patients compared with healthy controls, and to assess their relationship with renal function parameters.

Study Design: Comparative study

Place and Duration of Study: This study was conducted at the Department of Pharmaceutical Chemistry, College of Pharmacy, Ninevah University, Iraq from 15th April to 15th October 2025.

Methods: A total of 90 blood samples were collected and divided into three groups (n = 30 each): hemodialysis patients (G1), non-dialysis chronic kidney disease patients (G2), and healthy controls (G3). Serum homocysteine, malondialdehyde, and tumor necrosis factor-alpha levels were measured using enzyme-linked immunosorbent assay techniques, while glutathione peroxidase activity and renal function tests were determined by a colorimetric method.

Results: Serum homocysteine levels were significantly higher in the hemodialysis group (G1) compared with the control group, whereas significantly lower levels were observed in the non-dialysis chronic kidney disease group (G2). Malondialdehyde concentrations were significantly elevated in hemodialysis patients, indicating increased lipid peroxidation. In contrast, glutathione peroxidase activity was significantly reduced in both patient groups compared with controls. Serum tumor necrosis factor-alpha levels were significantly increased in both patient groups. Renal function markers, including urea and creatinine, were also significantly elevated, particularly in the hemodialysis group.

Conclusion: Oxidative stress and inflammation intensify with chronic kidney disease progression and dialysis, and suggest that homocysteine may play a key role in linking impaired renal function with redox imbalance in chronic kidney disease patients.

Key Words: Homocysteine, Tumor necrosis factor-alpha, glutathione peroxidase, Malondialdehyde, Enzyme-linked immunosorbent assay

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INTRODUCTION

Chronic kidney disease (CKD) represents a major global health burden and ischaracterized by a persistent decline in glomerular filtration rate (GFR) and/or structural kidney damage lasting more than three months. Beyond the progressive loss of renal excretory function, CKD is increasingly recognized as a complex systemic disorder involving metabolic dysregulation, oxidative imbalance, endothelial dysfunction, and chronic low-grade inflammation.[1,2]

One of the metabolic abnormalities frequently observed in CKD is hyperhomocysteinemia. Homocysteine is a sulfur-containing amino acid generated during methionine metabolism and normally undergoes remethylation to methionine or transsulfuration to cysteine. Impaired renal clearance, reduced enzymatic activity, and deficiencies in folate-dependent pathways,collectively contribute to elevated circulating

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homocysteine levels in CKD patients.[3] Increased homocysteine has been implicated in endothelial injury, vascular smooth muscle proliferation, and prothrombotic tendencies, thereby contributing to the markedly elevated cardiovascular risk observed in both dialysis and non-dialysis CKD populations.[4]

Oxidative stress plays a central mechanistic role in the progression of renal injury. It arises from an imbalance between reactive oxygen species (ROS) production and antioxidant defense systems. In CKD, multiple factors enhance ROS generation, including mitochondrial dysfunction, chronic inflammation, activation of the rennin-angiotensin system, and exposure to dialysis membranes in hemodialysis patients.[5] Elevated levels of lipid peroxidation products such as malondialdehyde (MDA) and altered activity of antioxidant enzymes, including glutathione peroxidase and superoxide dismutase, have been consistently reported in individuals with impaired renal function.[6]

Chronic inflammation is another hallmark of CKD. Tumor necrosis factor-alpha (TNF-α) is a key pro-inflammatory cytokine involved in immune modulation, apoptosis, and intracellular signaling pathways. Circulating TNF-α concentrations are frequently elevated in CKD patients and have been associated with declining renal function, protein-energy wasting, and cardiovascular morbidity.[7] In the context of hemodialysis, repeated blood–membrane interaction and complement activation further stimulate cytokine release, perpetuating a sustained inflammatory milieu.[8] Importantly, growing evidence supports a biological interplay between hyperhomocysteinemia, oxidative stress, and inflammatory cytokines. Elevated homocysteine can enhance oxidative stress through auto-oxidation and reduced nitric oxide bioavailability, which in turn activates redox-sensitive transcription factors such as nuclear factor-kappa B (NF-κB), promoting increased production of TNF-α and other inflammatory mediators.[9] This interconnected network may contribute to endothelial dysfunction and progressive renal damage. Therefore, simultaneous evaluation of homocysteine, oxidative stress markers, TNF-α, and renal function parameters may provide a more integrated understanding of the metabolic-inflammatory axis in both hemodialysis and non-dialysis CKD patients.[10]

METHODS

This comparative study was conducted at Qayyarah General Hospital, Iraq from 15th April to 15th October 2025. Sixty people between the ages of 20 and 65 who had been diagnosed with CKD were enlisted and divided into two groups: those receiving regular hemodialysis (G1, n=30) and those not receiving dialysis (G2, n=30). Thirty individuals who seemed to be in good condition and were matched for age and sex with the patient groups made up the control group (G3). There were a total of ninety competitors. Serum levels of homocysteine and MDA were determined by a competitive ELISA method. Measurement of TNF-α was performed using a sandwich ELISA technique based on antigen–antibody specific binding. Glutathione peroxidase activity was determined using a colorimetric method based on the Trinder reaction according to the principle described by Paglia and Valentine. In addition, serum urea, creatinine, and uric acid levels were measured using standard enzymatic colorimetric methods with commercially available diagnostic kits, following the manufacturer's instructions. The determination of urea was based on the urease-Berthelot reaction, creatinine was measured using the kinetic Jaffe method, and uric acid was determined by the uricase-peroxidase method.

Data were statistically analyzed using the Minitab software program (Version 17). Analysis of variance (ANOVA) was applied to assess significant differences among the studied groups. Mean values were compared using Duncan's Multiple Range Test at a significance level of $p \leq 0.05$. Pearson's correlation coefficient (r) was used to evaluate the relationship between plasma homocysteine levels and renal function biomarkers.

RESULTS

The significant biochemical changes associated with disease progression and dialysis and biochemical parameters, including homocysteine, MDA, GPx, TNF-α, and renal function markers. A significant ($P \leq 0.05$) elevation in serum homocysteine levels in hemodialysis patients (G1) compared to non-dialysis CKD patients (G2) and controls (G3). The average 819.8±34.1 ng/ml in group G1, 492.0±55.4 ng/ml in G2 and 573.0±85.6 ng/ml in the control group (G3) respectively. This increase can be attributed to impaired renal clearance of homocysteine and dysfunction of its metabolic pathways, particularly remethylation and trans-sulfuration, which become more pronounced with progressive renal impairment. Serum malondialdehyde levels were significantly higher ($P \leq 0.05$) while the distribution pattern among groups in dialysis patients compared to non-dialysis patients and healthy groups. The mean values were 20.78±5.35 ng/ml in group G1, 10.73±4.66 ng/ml and 10.48±4.03 ng/ml in the control group (G2, G3) respectively indicating enhanced lipid peroxidation and increased oxidative stress. Regarding antioxidant status, glutathione peroxidase (GPx) activity was significantly reduced in both patient groups compared with the control group. GPx activity decreased by approximately 33%, with mean values was 56.94±9.84 IU/L in the first group and 58.47±7.22 IU/L in the second group, versus 166.67±5.06 IU/L in healthy controls (Table 1, Figs. 1-3).

The average level of urea were 116.67±8.71 mg/dl in G1, 75.47±6.04 mg/dl in G2, and 32.97±5.69 mg/dl in the control group (G3). The creatinine levels were

7.02±2.39 mg/dl, 6.07±1.31 mg/dl, and 1.05±0.12 mg/dl, respectively. Both urea and creatinine concentrations were significantly higher in hemodialysis patients (G1) and non-dialysis CKD patients (G2) compared with controls ($p \leq 0.05$), with the highest levels observed in the dialysis group, indicating progressive impairment of renal function. The mean serum uric acid was 4.521±1.159 mg/dl in G1, 5.747±0.604 mg/dl in G2, and 4.193±0.418 mg/dl in G3. Unlike urea, creatinine and uric acid levels were significantly elevated in not dialysis CKD patients (G2) ($p \leq 0.05$) but decreased in hemodialysis patients (G1) compared to controls ($p \leq 0.05$). Overall, the findings of the present study indicate a clear association between impaired renal function, oxidative stress, and inflammation in patients with chronic kidney disease.

Table No. 1: Biochemical parameters in hemodialysis, non-dialysis CKD patients and controls

Variable	G1	G2	G3	P value
Homocysteine (ng/mL)	819.8±34.1	492.0±55.4	573.0±85.6	0.01*
MDA (ng/mL)	20.78±5.35	10.73±4.66	10.48±4.03	0.01*
GPx (IU/L)	56.94±9.84	58.47±7.22	166.67±5.06	0.01*
TNF-α (Pg/mL)	22.99±1.48	24.96±5.05	8.64±0.83	0.001*
Urea (mg/dl)	116.67±8.71	75.47±6.04	32.97±5.69	0.01*
Creatinine (mg/dl)	7.017±2.390	2.373±1.31	1.052±0.12	0.01*
Uric acid (mg/dl)	4.521±1.159	6.070 ± 0.604	5.12±0.418	0.01*

*P ≤ 0.05 (Significant)

Table No. 2: Correlation between homocysteine and selected biochemical parameters in study groups

Parameters	G1	G2	G3
MDA	0.235	0.471	0.616
GPx	- 0.104	0.323	0.431
TNF-α	- 0.115	0.203	- 0.06
Urea	- 0.203	-0.234	0.382
Creatinine	- 0.044	-0.329	0.2
Uric acid	0.283	-0.003	0.064

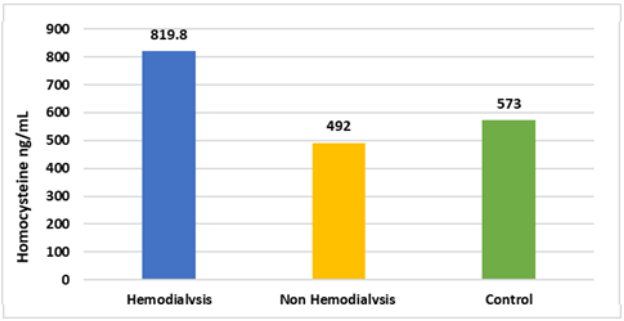

Figure No. 1: Homocysteine levels

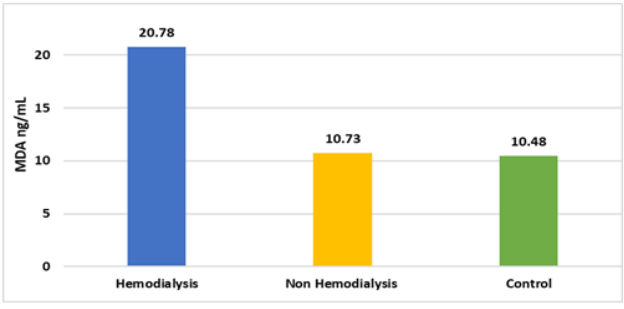

Figure No. 2: Malondialdehyde levels

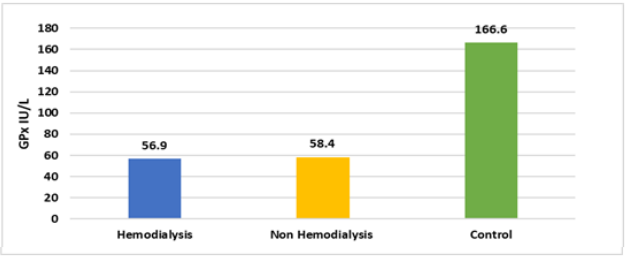

Figure No. 3: Glutathione peroxidase levels

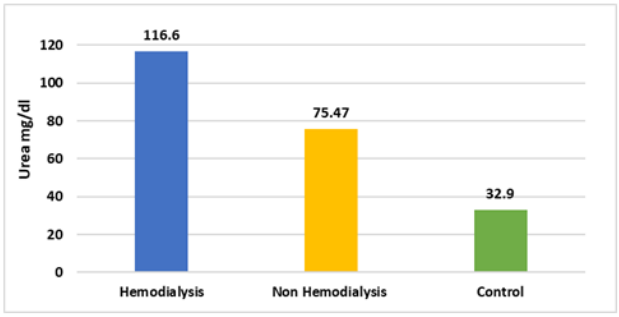

Figure No. 4: Urea levels

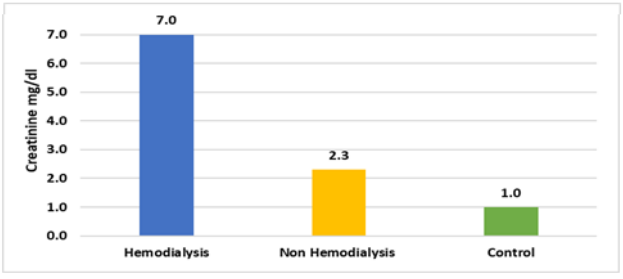

Figure No. 5: Creatinine levels

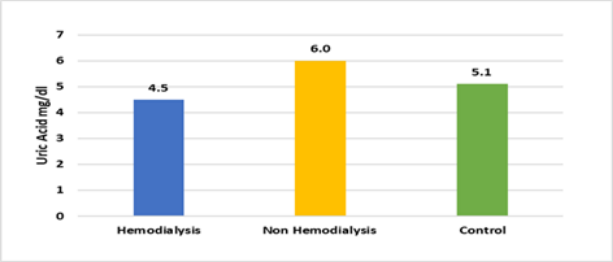

Figure No. 6: Uric acid levels

The significant elevation in serum homocysteine, MDA, TNF-α, urea, and creatinine levels, together with the marked reduction in GPx activity, suggests that CKD patients experience increased oxidative stress and inflammatory responses accompanied by diminished antioxidant defense mechanisms. The correlation analysis revealed a positive association between serum homocysteine and MDA levels across all studied groups, with the strongest correlation observed in the control group (Table 2, Figs. 4-6).

DISCUSSION

Hyperhomocysteinemia is a well-recognized metabolic disturbance in CKD and has been shown to worsen as glomerular filtration rate declines, reaching its highest levels in patients undergoing hemodialysis.[11] The markedly elevated homocysteine concentrations observed in the hemodialysis group are consistent with previous reports indicating that dialysis is insufficient to fully normalize homocysteine levels, despite partial removal during treatment sessions. In addition, uremic toxicity and deficiencies of folate and B-complex vitamins may further disrupt homocysteine metabolism in these patients. Several studies have highlighted a strong association between elevated homocysteine levels and increased cardiovascular risk in patients with end-stage renal disease.[12] While patients with CKD not on dialysis showed significantly lower plasma homocysteine levels compared to dialysis patients and healthy individuals, this may be attributed to strict adherence to low-protein diets aimed at slowing the decline in kidney function, as well as variations in dietary intake and vitamin levels that affect homocysteine metabolism. Nevertheless, elevated homocysteine levels remain a clinically important biomarker in CKD, given their strong association with endothelial dysfunction and an increased risk of cardiovascular disease.[13,14]

Serum malondialdehyde supports the concept that oxidative stress intensifies in advanced stages of kidney disease, particularly in patients receiving hemodialysis. Elevated MDA levels in this group may result from chronic inflammation, accumulation of uremic toxins, and repeated exposure to bioincompatible dialysis membranes, all of which promote excessive generation of reactive oxygen species.[15] In contrast, no significant difference in MDA levels was detected between non-dialyzed CKD patients and healthy controls, suggesting that oxidative damage becomes more evident in later stages of renal dysfunction. Similar findings have been reported in previous studies, which demonstrated that markers of oxidative stress are markedly elevated mainly in patients with end-stage renal disease rather than in those with early or moderate CKD.[16] The significantly higher malondialdehyde levels observed in hemodialysis patients compared with non-dialyzed CKD patients may be attributed to dialysis-induced oxidative stress, as previously reported by Durak et al.[17]

Glutathione peroxidase reduction reflects a substantial impairment of the antioxidant defense system in patients with chronic kidney disease. The decreased GPx activity may be attributed to selenium deficiency, reduced enzymatic synthesis, and/or oxidative inactivation under uremic conditions. Consistent with the present findings, previous studies have reported significantly diminished GPx activity in both non-dialyzed CKD and hemodialysis patients, supporting the concept of a disrupted redox balance characterized by excessive oxidant generation and weakened antioxidant capacity.[18,19]

The absence of a significant difference in GPx activity between hemodialysis and non-dialyzed patients suggests that impairment of antioxidant defenses may occur early in the course of chronic kidney disease and persist throughout disease progression. This sustained reduction in antioxidant capacity may increase susceptibility to oxidative injury, endothelial dysfunction, and subsequent cardiovascular complications.

Overall, the concurrent elevation of homocysteine and MDA levels, together with the reduction in GPx activity observed in the present study, supports the hypothesis that oxidative stress and impaired antioxidant defense play a central role in the pathophysiology of chronic kidney disease. These alterations appear to be more pronounced in hemodialysis patients, highlighting the contribution of dialysis-related factors to oxidative imbalance and systemic inflammation.[20]

Elevated levels of tumor TNF-α in patients with CKD and those undergoing hemodialysis are primarily attributed to the persistent inflammatory state associated with renal dysfunction. CKD is increasingly recognized as a condition characterized by chronic low-grade systemic inflammation resulting from the accumulation of uremic toxins, oxidative stress, and immune system dysregulation. These factors stimulate immune cells, particularly monocytes and macrophages, to produce higher levels of pro-inflammatory cytokines such as TNF-α. Consequently, circulating TNF-α concentrations in CKD patients are significantly higher than those observed in healthy individuals.[21,22]

Additionally, inflammatory cytokines including TNF-α have been shown to be strongly associated with the presence and severity of chronic kidney disease. Clinical studies demonstrated that serum TNF-α levels are significantly higher in CKD patients compared with healthy controls and are independently associated with reduced glomerular filtration rate and increased albuminuria.[23]

The high increase in the renal functional indicators, especially, serum urea and creatinine, in patients under hemodialysis can be explained by the existence of advanced chronic kidney disease with a severe decrease in the level of glomerular filtration rate (GFR). The functional loss of nephrons with time causes functional impairment in the clearance of nitrogenous wastes products by the kidney, which ultimately makes it find its way into the circulatory system. Even though these metabolites are partially cleared by hemodialysis, they are not fully cleared and build up in between dialytic sessions which lead to continuously high levels. Moreover, higher protein metabolism, persistent inflammation and metabolic alterations that are likely to accompany end-stage renal disease can also contribute to urea levels, although serum creatinine is a dependable measure of reduced renal clearance capacity and disease progression in patients on dialysis.[24]

Serum uric acid reflects the partial clearance of uric acid during dialysis sessions, whereas the kidneys decreased ability to excrete uric acid in non-dialysis CKD leads to its accumulation. Elevated uric acid in CKD has important clinical implications, as they contribute to oxidative stress, hypertension, and further progression of renal dysfunction.[25]

The alterations may contribute to the progression of renal dysfunction and related complications, particularly in hemodialysis patients. The correlation analysis revealed a positive association between serum homocysteine and malondialdehyde (MDA) levels across all studied groups, with the strongest correlation observed in the control group (Table 2). This finding suggests a close link between hyperhomocysteinemia and oxidative stress, as elevated homocysteine levels are known to promote the generation of reactive oxygen species and lipid peroxidation.[26] In contrast, an inverse relationship was observed between homocysteine and GPx activity in the patient groups, indicating that increased homocysteine levels may be associated with impaired antioxidant defense mechanisms. This reduction in GPx activity could contribute to the accumulation of oxidative damage in CKD patients.[27]

The relationship between homocysteine and TNF-α varied among the groups, reflecting the complex interaction between metabolic disturbances and inflammatory pathways in CKD. The observed alterations suggest that homocysteine may indirectly influence inflammatory responses through oxidative stress-mediated mechanisms.[28]

Furthermore, weak and inconsistent correlations were found between homocysteine and renal function markers (urea, creatinine, and uric acid), which may indicate that homocysteine levels are influenced not only by renal impairment but also by additional metabolic and nutritional factors.[29]

Overall, these findings support the hypothesis that homocysteine plays a central role in linking oxidative stress and inflammation in CKD, particularly in patients undergoing hemodialysis.[30]

CONCLUSION

Patients with chronic kidney disease, particularly those undergoing hemodialysis, exhibit significant alterations in several biochemical and inflammatory markers compared with healthy controls. Serum homocysteine, malondialdehyde, tumor necrosis factor-alpha, urea, and creatinine levels were significantly elevated, whereas glutathione peroxidase activity was significantly reduced. The enhanced oxidative stress, inflammation, and impaired antioxidant defense mechanisms in chronic kidney disease patients.

Author's Contribution:

Concept & Design or acquisition of analysis or interpretation of data:	Sabri Ali Sultan, Fadhel D. Khaled
Drafting or Revising Critically:	Sabri Ali Sultan, Yasser A.M Sulaiman
Final Approval of version:	All the above authors
Agreement to accountable for all aspects of work:	All the above authors

Conflict of Interest: The study has no conflict of interest to declare by any author.

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