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Role of Clinical Chemistry in Hemodialysis: Assessment of Biochemical Markers and Electrolyte Imbalances

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Abstract. Background; Chronic kidney disease (CKD) and renal failure are significant global health concerns, affecting millions of individuals and contributing to high morbidity and mortality rates. Aims of the study; This study aims to assess biochemical, inflammatory, and oxidative stress markers in renal failure patients to identify biomarkers and guide therapeutic strategies. Methodology; This case-control study, conducted between January 1, 2023, and January 1, 2024, included 100 renal failure patients and 50 healthy controls, aged 35-55 years. The participants' gender distribution was 60% male and 40% female in the patient group, and 50% male and 50% female in controls. Renal function parameters (serum creatinine, BUN, GFR, urine output) were measured using an automated chemistry analyzer. Biochemical markers (potassium, calcium, phosphorus, PTH) were assessed with an electrolyte analyzer and immunoassay techniques. Inflammatory markers (CRP, TNF-α) and oxidative stress markers (MDA, GPx) were quantified using ELISA and spectrophotometric methods. Result; This study compared 100 renal failure patients and 50 healthy controls. Age differences were significant ($p=0.045$), with the renal failure group being older (53.2 ± 12.5 years). Gender, occupation, marital status, and smoking history showed no significant differences. Renal function parameters revealed significant differences: serum creatinine (7.4 ± 3.2 mg/dL vs. 0.9 ± 0.2 mg/dL, $p<0.001$), BUN (55.2 ± 19.1 mg/dL vs. 12.3 ± 4.5 mg/dL, $p<0.001$), GFR (15.6 ± 8.3 mL/min vs. 95.3 ± 10.2 mL/min, $p<0.001$), and urine output (450 ± 230 mL/day vs. 1600 ± 200 mL/day, $p<0.001$). Biochemical markers like potassium, calcium, phosphorus, and PTH also showed significant differences. Inflammatory and oxidative markers (CRP, TNF-α, MDA, GPx) had notable disparities, all with significant p-values. Conclusions; This study demonstrates significant differences between renal failure patients and healthy controls in renal function, biochemical markers, and inflammation. Renal failure patients had higher serum creatinine, BUN, and phosphorus levels, along with altered potassium, calcium, and PTH levels. Elevated inflammatory markers (CRP, TNF-α) and oxidative stress markers (MDA, GPx) suggest enhanced inflammation and oxidative stress

Highlights:

1. CKD study: Renal function, biochemical, inflammatory, oxidative stress markers analyzed.
2. Significant differences in creatinine, BUN, GFR, CRP, TNF-α observed.
3. Results highlight inflammation, oxidative stress in renal failure patients.

Keywords: Renal failure, Biochemical markers, Inflammation, Oxidative stress, Serum creatinine, Parathyroid hormone

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Introduction

Chronic kidney disease (CKD) patients who are treated with renal replacement therapy (RRT) by hemodialysis (HD) face a number of issues that are difficult to manage. The kidneys are responsible for many functions that are critical to homeostasis, and as renal function declines, patients are more likely to develop a variety of metabolic disorders. The most frequent metabolic issue is dyselectrolitemia – the development of electrolyte or acid-base balance abnormalities (Timofte et al., 2021). The mortality of dialysis patients is linked to the progression of dyselectrolitemia. Unfortunately, in-patient hospital settings and maintenance hemodialysis (MHD) result in a patient population that often has poorer metabolic control in terms of serum electrolyte levels and other biochemical markers (Hu et al., 2024; Pang and Meng,, 2022). Hemodialysis is the most widely used treatment for end-stage renal disease (ESRD) patients. In hemodialysis, blood is taken from the patient and passed through a dialyzer – a membrane tube that separates blood and dialysate. Mid-sized molecules that are uremic toxins are removed by diffusion, and electrolytes are removed based on the composition of the dialysate. The dialysate solution is usually prepared from purified water, and electrolytes are added to it to match normal plasma levels. Water is also removed from the blood; osmotic pressure decreases due to adding sodium to the dialysate, and water moves from the blood to the dialysate side in order to match osmotic pressures (Caturano et al., 2024; Zhu et al., 2024). Clinical chemistry is the science of analyzing blood and urine for the diagnosis and treatment of disease. It handles the processing, handling, transportation and storage of biological samples, especially blood and serum, to evaluate various biochemical parameters. It plays a key role in the management of patients on chronic hemodialysis. Kidney disease can be categorized into acute or chronic. Acute Kidney Injury (AKI) may be reversible with the management of its causative factors. On the other hand, Chronic Kidney Disease (CKD) is progressive and irreversible deterioration in renal function due to the pathological damage of nephrons (Kim et al., 2022; Suzuki et al., 2021). Stage V CKD (end-stage renal disease/ESRD) is the last stage which requires renal replacement therapy (RRT) for long-term survival. Hemodialysis is the most commonly used method of RRT, where blood is filtered through a dialyzer, an artificial kidney, to remove the accumulated uremic toxins, extra fluid, and to maintain the electrolyte and acid-base balance. In the absence of kidney function, the

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dialysis therapy goals are to mimic the kidney function as much as possible by removing the uremic toxins and maintaining the homeostasis of various biochemical parameters (Kim et al., 2022; Suzuki et al., 2021; Heredia-Martinez et al., 2022). The kidneys play a key role in maintaining the body's homeostasis. Chronic kidney disease (CKD) is a progressive and permanent decline of kidney function, resulting in the accumulation of toxic metabolic byproducts and fluid, electrolyte, and acid-base imbalance (Timofte et al., 2021). Hemodialysis (HD) is a widely used renal replacement therapy (RRT) method in patients with end-stage kidney disease (ESKD) that can perform some key functions of the kidney, such as filtration and excretion (Timofte et al., 2021; Mehmood et al., 2022; Ajam., 2020). Hepatic and Renal functions were assessed by estimated biochemical levels of Bilirubin, Urea, Creatinine, ALP, ALT, and AST. There is no significant association between gender and all biochemical markers assessed in this study. Fifty (42.0%) patients were having Urea levels >60 mg/dL and 69 (58.0%) patients were having Urea levels ≤ 60 mg/dL. Urea levels were higher in patients taking an antihypertensive drug as compared to those not taking antihypertensive drugs with a p-value of 0.03887 which suggests a significant association between Urea levels and antihypertensive drug consumption (Faleh Hassen et al., 2018). Fifty-one (43.0%) patients were having Creatinine levels > 8 mg/dL and Sixty-nine (57.0%) patients were having Creatinine levels ≤ 8 mg/dL. Creatinine levels were higher in patients taking an antihypertensive drug as compared to those not taking antihypertensive drugs with a p-value of 0.02692 which suggests a significant association between Creatinine levels and Antihypertensive drug consumption (Laville et al.2023; Yang et al., 2020; Shen et al., 2022). Almost all studies on the control of calcium and phosphate balance during hemodialysis include a mathematical description of the mass transfer of these solutes across the dialyzer membrane and through the arterial and venous blood compartments. There are also clinical studies seeking to optimize calcium and phosphate control during hemodialysis, some of which are in direct contradiction with one another (Carvalho Barreto et al., 2019; Boamah, 2020; Laursen, 2020; Pstras et al.2022). During routine hemodialysis, sodium (Na+) and potassium (K+) levels are checked via clinical chemistry tests 15 minutes, 30 minutes, and at the end of the treatment. Changes in these electrolyte levels can lead to serious consequences. Na+ and K+ are actively regulated ions in the body and need to be assessed regularly. Sodium concentration in the body is

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maintained by the kidney through reabsorption of sodium in nephrons. In patients with kidney failure, sodium cannot be excreted, causing hypernatremia. Patients who are hypernatremic may experience hypertension, edema, and heart failure. To manage Na+ levels during hemodialysis, Na+ in the dialysate is fixed at 137mEq/L. If the patient's serum Na+ is greater than 138mEq/L, sodium profiling may be conducted. Sodium profiling involves gradually decreasing the dialysate sodium concentration to treat hypernatremia. Conversely, if serum sodium is lower than 134mEq/L, it could lead to cerebral edema, which can be life threatening. Thus careful handling is required when serum sodium is less than 124mEq/L (Fidan and Ağırbaş, 2023; Wu et al.2020; Yildiz et al.2023). Calcium, phosphate, and vitamin D are essential minerals for skeletal homeostasis. In chronic kidney disease (CKD), calcium and phosphate mass balance alterations can happen due to abnormalities in excretion and/or absorption. Hemodialysis (HD) patients present complex alterations in mineral and parathormone metabolic system due to CKD status, systemic abnormalities, medications, and dialysis procedures. These alterations influence bone health and the development of extra-skeletal calcifications, particularly in cardiovascular structures, causing increased morbidity and mortality. Abnormalities in serum calcium and phosphate concentrations can induce tissue calcification or osteopenia due to loss of skeletal calcium reserves (M. Elias et al., 2021; Al-jumaili and Al-Jumaili., 2024; Maranduca et al.2024; Pazianas and Miller, 2020).

Methods

This case-control study was conducted between January 1, 2023, and January 1, 2024, involving 100 renal failure patients and 50 healthy controls. The participants' mean age was 35 to 55 years for patients and controls, with a gender distribution of 60% male and 40% female among patients and an even 50% male and 50% female distribution in controls. Renal function parameters, including serum creatinine, blood urea nitrogen (BUN), glomerular filtration rate (GFR), and urine output, were measured using an automated chemistry analyzer. Biochemical markers such as serum potassium, calcium, phosphorus, and parathyroid hormone (PTH) were assessed using an electrolyte analyzer and immunoassay techniques like chemiluminescence. Inflammatory markers, including C-reactive protein (CRP) and tumor necrosis factor-alpha (TNF-α), were quantified using enzyme-linked immunosorbent assay (ELISA). Oxidative stress markers,

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malondialdehyde (MDA) and glutathione peroxidase (GPx), were evaluated using spectrophotometric methods.

Statistical analysis:

Statistical analysis plays a crucial role in examining quantitative data, offering methods for describing data and conducting straightforward inferences for both continuous and categorical variables. This process involves data collection and testing the relationship between two statistical datasets. In this study, data were presented as frequencies and percentages. Statistical analysis was performed using SPSS software (version 26). For variables with a normal distribution, the dependent t-test (two-tailed) and independent t-test (two-tailed) were applied. For variables that did not follow a normal distribution, the Mann-Whitney U test, Wilcoxon test, and Chi-square test were used. A p-value of less than 0.05 was considered statistically significant.

Ethical approval:

Ethical approval for this study was obtained at Al-Imamain Alkadhimain Medical City Hospital. The participants were informed about the study and verbal consent was obtained prior to sample collection.

Result and Discussion

Sociodemographic Characteristics of Renal Failure Patients and Healthy Controls

The results showed sociodemographic differences between patients with renal failure (n=100) and healthy controls (n=50). Age analysis showed that the mean age in the renal failure group was 53.2 ± 12.5 years, while in the healthy group it was 47.8 ± 13.4 years, with a significant difference between the two groups (p=0.045). As for gender, the percentage of males in the patient and healthy groups was similar (60% for patients and 58% for healthy), indicating no significant difference between the two groups (p=0.85). As for occupation, the percentage of workers in the patient group was 30% compared to 50% in the healthy group, but the difference was not significant (p=0.15). In the case of marital status, 70% of renal failure patients were married, while the percentage was 68% in the healthy group, with no significant difference between the two groups (p=0.72). Regarding smoking history, 40% of patients with renal failure were smokers, compared to 35% in the healthy group, without a significant difference (p=0.64).

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Table 1: Sociodemographic Characteristics of Participants

Characteristic	Renal Failure Patients (n=100)	Healthy Controls (n=50)	p-value
Age (Mean ± SD)	53.2 ± 12.5 years	47.8 ± 13.4 years	0.045
Gender (Male/Female)	60% Male / 40% Female	50% Male / 50% Female	0.85
Occupation	30% Employed / 70% Unemployed	50% Employed / 50% Unemployed	0.15
Marital Status	70% Married / 30% Single	68% Married / 32% Single	0.72
Smoking History	40% Smokers / 60% Non-smokers	35% Smokers / 65% Non-smokers	0.64

Comparison of Renal Function Parameters Between Renal Failure Patients and Healthy Controls

Results show differences between patients with renal failure (n=100) and healthy controls (n=50) in terms of renal function parameters. Serum creatinine levels in patients with renal failure were 7.4 ± 3.2 mg/dL, while in healthy controls they were 0.9 ± 0.2 mg/dL, with a strongly significant difference between the two groups ($p<0.001$). As for blood urea nitrogen (BUN) levels, patients had 55.2 ± 19.1 mg/dL, while healthy controls had 12.3 ± 4.5 mg/dL, also with a significant difference ($p<0.001$). For glomerular filtration rate (GFR), patients with renal failure had 15.6 ± 8.3 ml/min, while healthy controls had 95.3 ± 10.2 ml/min, with a significant difference ($p<0.001$). Finally, the mean urine output in patients with renal failure was 450 ± 230 ml/day, while in healthy controls it was 1600 ± 200 ml/day, with a significant difference between the two groups ($p<0.001$).

Table 2: Renal Function Parameters

Parameter	Renal Failure Patients (n=100)	Healthy Controls (n=50)	p-value
Serum Creatinine (mg/dL)	7.4 ± 3.2	0.9 ± 0.2	< 0.001
Blood Urea Nitrogen (BUN, mg/dL)	55.2 ± 19.1	12.3 ± 4.5	< 0.001
Glomerular Filtration Rate (GFR, mL/min)	15.6 ± 8.3	95.3 ± 10.2	< 0.001
Urine Output (mL/day)	450 ± 230	1600 ± 200	< 0.001

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Comparison of Biochemical Markers Between Renal Failure Patients and Healthy Controls

The results show differences between patients with renal failure (n=100) and healthy controls (n=50) in some biochemical indicators. Serum potassium levels in patients with renal failure were 5.8 ± 1.3 mmol/L, while in the healthy group they were 4.2 ± 0.5 mmol/L, with a significant difference between the two groups (p=0.002). As for serum calcium levels, they were 8.1 ± 1.2 mg/dL in patients with renal failure, while in healthy controls they were 9.4 ± 0.7 mg/dL, with a significant difference (p<0.001). As for phosphorus, the levels in patients with renal failure were 5.1 ± 2.2 mg/dL, while in healthy controls they were 3.2 ± 1.1 mg/dL, with a significant difference as well (p=0.001). Finally, the parathyroid hormone (PTH) levels in patients with renal failure were 350 ± 190 pg/ml, while in healthy controls they were 50 ± 20 pg/ml, with a significant difference (p<0.001).

Table 3: Biochemical Markers

Marker	Renal Failure Patients (n=100)	Healthy Controls (n=50)	p-value
Serum Potassium (mmol/L)	5.8 ± 1.3	4.2 ± 0.5	0.002
Serum Calcium (mg/dL)	8.1 ± 1.2	9.4 ± 0.7	< 0.001
Phosphorus (mg/dL)	5.1 ± 2.2	3.2 ± 1.1	0.001
Parathyroid Hormone (PTH, pg/mL)	350 ± 190	50 ± 20	< 0.001

Comparison of Inflammatory and Oxidative Stress Markers Between Renal Failure Patients and Healthy Controls

The results showed differences between patients with renal failure (n=100) and healthy controls (n=50) in some inflammatory and oxidative markers. The levels of C-reactive protein (CRP) in the serum of patients with renal failure were 11.6 ± 5.2 mg/L, while in the healthy group it was 2.3 ± 1.1 mg/L, with a strong significant difference between the two groups (p<0.001). As for the levels of tumor necrosis factor-alpha (TNF-α), it was 35.3 ± 15.2 pg/mL in patients with renal failure, while it was 10.1 ± 4.2 pg/mL in the healthy group, with a significant significant difference (p<0.001). As for the levels of malondialdehyde (MDA), they were 6.8 ± 3.4 µmol/L in the serum of

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patients with renal failure, while they were 3.5 ± 1.1 µmol/L in the healthy group, with a significant difference (p=0.002). Finally, the levels of glutathione peroxidase (GPx) in patients with renal failure were 120 ± 20 units/ml, while they were 185 ± 30 units/ml in healthy subjects, with a clear significant difference (p<0.001).

Table 4: Inflammatory and Oxidative Stress Markers

Marker	Renal Failure Patients (n=100)	Healthy Controls (n=50)	p-value
C-Reactive Protein (CRP, mg/L)	11.6 ± 5.2	2.3 ± 1.1	< 0.001
Tumor Necrosis Factor-alpha (TNF-α, pg/mL)	35.3 ± 15.2	10.1 ± 4.2	< 0.001
Malondialdehyde (MDA, µmol/L)	6.8 ± 3.4	3.5 ± 1.1	0.002
Glutathione Peroxidase (GPx, U/mL)	120 ± 20	185 ± 30	< 0.001

Discussion:

Renal failure is a complex clinical condition with progressive loss of kidney function resulting in derangement of metabolic, inflammatory and oxidative pathways. When the kidneys stop working, metabolic waste and toxins build up in the body, leading to a cascade of inflammation and oxidative stress that promotes organ damage in a systemic manner. Biochemical, inflammatory, and oxidative stress marker alterations play an essential role in understanding the pathophysiological mechanisms of renal failure and potential therapeutic targets. Many of these markers have been investigated, yielding useful information on disease progression but also on the variability of findings across different patient populations, disease stages, and management strategies (Jassim et al., 2024; Hassen et al., 2024). As is often the case with renal failure, the parameters of renal function in our controlled study show similarities with a number of studies but area also at variance with others. The higher serum creatinine levels in the patients (7.4 ± 3.2 mg/dL) than in the controls (0.9 ± 0.2 mg/dL) are in agreement with those of (Hawale et al., 2021), reported that decreased vigor glomerular filtration rate GFR causes accumulation of creatinine (Lim et al., 2021). However, Rodrigues et al., 2018), noted variable creatinine levels between patients due to differences in age, sex, and

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disease progression. Likewise, the extremely high levels of BUN (55.2 ± 19.1 mg/dL in patients and 12.3 ± 4.5 mg/dL in controls) correlate with (Lee et al., 2021), focusing on links to impaired nitrogen excretion. In contrast (Joliansyah et al., 2017), relatively lower BUN levels in patients who received regular dialysis, confirming treatment effects. The marked decrease in GFR (15.6 ± 8.3 mL/min in patients vs. 95.3 ± 10.2 mL/min in matched controls) is consistent with the report by (Kaczkan et al., 2021), solidifying its position as a primary renal function marker (Sabouri et al., 2023), found fluctuating GFR according to chronicity. Reduced urine output (450 ± 230 mL/day in patients vs. 1600 ± 200 mL/day in controls) concurs with (Sahathevan et al., 2020), who found an association between oliguria and severe acute renal failure, but (Ma et al., 2020), noted milder declines in some patients, suggesting it was due to early stages of the disease. These discrepancies might result from differences in study populations, disease stages, or interventions (e.g., dialysis), emphasizing the complexity of renal failure and the importance of personalized patient-based evaluation. The analysis of biochemical markers shows marked differences in patients with renal failure compared to healthy controls, in line with previous related studies but deviating in some respects. Serum potassium levels above normal (5.8 ± 1.3 mmol/L in patients vs 4.2 ± 0.5 mmol/L in controls, $p = 0.002$) echo the findings of (de Rooij et al., 2022), who associated hyperkalemia with reduced renal excretory ability in chronic kidney disease (CKD). However, (Shibata et al., 2022), observed that milder elevations in potassium were associated with dietary potassium restrictions or medications, suggesting treatment effects. In agreement with (Debowska et al., 2020), the serum calcium levels were significantly reduced in patients (8.1 ± 1.2 mg/dL vs. 9.4 ± 0.7 mg/dL in controls, $p < 0.001$) (2018), which ascribed hypocalcemia as a result of impaired vitamin D metabolism and secondary hyperparathyroidism in CKD. Conversely, (Nakamura et al., 2022), reported differential calcium levels based on vitamin D supplementation. Higher phosphorus values in patients (5.1 ± 2.2 mg/dL vs. 3.2 ± 1.1 mg/dL in controls, $p = 0.001$) are additional supportive of (Guedes et al., 2023), who emphasized diminished renal phosphorus excretion as a central mechanism in CKD. Finally, high PTH (350 ± 190 pg/mL in patients vs. 50 ± 20 pg/mL in controls, $p < 0.001$), validated the findings of (Wang et al., 2024), highlighted that secondary hyperparathyroidism as a consequence of imbalance of calcium-phosphorus metabolism in patients with CKD.

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Variations in dietary management, stage of renal failure, and the use of phosphate binders or vitamin D analogs likely explain discrepancies between studies and highlight the complex interplay of factors that affect biochemical derangements in renal failure. Renal dysfunction affects the whole organism, and the difference in inflammatory and oxidative stress markers between patients and controls represents a clear highlight of this fact. Elevated C-reactive protein (CRP) levels in patients (11.6 ± 5.2 mg/L vs controls 2.3 ± 1.1 mg/L, $p < 0.001$) concur with data by (Valga et al., 2022), which suggested that increasing concentrations of uremic toxins and oxidative stress are responsible for accelerating chronic inflammation. Likewise, tumor necrosis factor-alpha (TNF-α) levels were significantly elevated in patients (35.3 ± 15.2 pg/mL vs. 10.1 ± 4.2 pg/mL in controls, $p < 0.001$), in keeping with (Amalia et al., 2022), noting that TNF-α induces inflammation and endothelial dysfunction in chronic kidney disease (CKD). Regarding oxidative stress, the authors found a significantly higher level of the lipid peroxidation marker malondialdehyde (MDA) in patients (6.8 ± 3.4 µmol/L vs. 3.5 ± 1.1 µmol/L in controls, $p = 0.002$), confirming the findings of (Liu et al., 2024), which associated elevated MDA levels with oxidative damage as a consequence of impaired antioxidant defenses in CKD. In turn, glutathione peroxidase (GPx) activity was significantly lower in patients (120 ± 20 U/mL vs. 185 ± 30 U/mL in controls, $p < 0.001$), which associated with the findings by (Barroso et al., 2024), finding decreased GPx activity associated with a reduced antioxidant capacity in CKD. Interstudy variability in markers levels may be the result of differences in patient demographics, stages of CKD and types of interventions (e.g. antioxidant supplementation). These findings suggest that inflammation and oxidative stress are intertwined risk factors for CKD progression and that they might be considered therapeutic targets.

Conclusion

In conclusion, this study highlights significant differences in various sociodemographic, renal function, biochemical, inflammatory, and oxidative stress markers between renal failure patients and healthy controls. The renal failure patients exhibited markedly higher serum creatinine, BUN, and phosphorus levels, along with significantly lower GFR and urine output compared to healthy controls, indicating impaired renal function. Biochemical markers such as serum potassium, calcium, and

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parathyroid hormone also showed significant alterations, reflecting disturbances in mineral metabolism. Additionally, inflammatory markers like CRP and TNF-α, along with oxidative stress indicators such as MDA and GPx, were significantly elevated in renal failure patients, suggesting a heightened inflammatory and oxidative state associated with renal dysfunction. These findings underline the importance of monitoring renal function and biochemical markers in renal failure patients, which could aid in early diagnosis, treatment, and the prevention of complications. Further studies are needed to explore the potential therapeutic implications of targeting inflammation and oxidative stress in renal failure management.

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