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Studying of Uropathogenic Bacteria and Selective Immunological Markers (suPAR, CFH and FGF 23) in Hemodialysis Patients with Renal Failure

A Thesis

Submitted to the Council of the College of Medicine/ University of Kerbala in Partial Fulfillments of the Requirements for the Degree of Master in Medical Microbiology

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بِسْمِ ٱللَّهِ ٱلرَّحْمَٰنِ ٱلرَّحِيمِ

(فَتَعَالَى ٱللَّهُ ٱلْمَلِكُ ٱلْحَقُّ وَلَا تَعْجَلْ
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صدق الله العلي العظيم

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Supervisors Certification

We certify that this M.Sc. thesis titled:

Studying of Uropathogenic Bacteria and Selected Immunological Markers (FGF 23, CFH and suPAR) in Hemodialysis Patients with Renal failure

Was prepared under our supervision in the College of Medicine/ University of Karbala, as a partial fulfillment of the requirements for the Degree of Master of Science in Medical Microbiology.

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We have examined the student (Ahmed Hassan Kassar Humaidi) in its contents. In our opinion, it meets the standards of a thesis for the degree of Masters in Medical Microbiology.

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Dedication

I dedicate this work

To……

The first teacher, our Prophet Mohammed (may Allah bless him and his family).

The soul of my father

My mother.

My brothers, sisters

my friends.

To those who lighted the way for me, my teachers

Ahmed

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Ahmed

Summary

Infection is a common complication and it is the second leading cause of death in hemodialysis patients. Renal failure (RF) compromises immune defenses and increases susceptibility to infections. Biomarkers such as Complement Factor H (CFH), Soluble urokinase plasminogen activator recopter (suPAR) and Fibroblast Growth Factor 23 (FGF23) have been implicated in renal pathophysiology.

The study aims to characterize and distribute types of bacterial infection that cause urinary tract infection in hemodialysis patients and to evaluate some immunological markers and other parameters in renal failure patients.

The present study is designed to deal with hemodialysis patients, after establishing the diagnosis by a physician and clinical diagnosis at the dialysis center in Imam Al-Hussein Hospital in Karbala province, during the period from October 2024 to January 2025.

A total of 120 blood samples were collected from participants (90 patients and 30 healthy controls), the patient group was sub subdivided into :30 patients with renal failures and UTI, 30 patients with renal failures and without UTI, and 30 patients with UTI and without renal failures , compared with the healthy control group. The age group varied from 10-80 years. urine culture was done for bacterial isolation and identification. Blood samples were collected to measure immunological markers (FGF 23, CFH, suPAR). After that, many analyses were performed, including kidney function test (urea, creatinine), hematological, electrolyte, and parathyroid hormone (PTH) parameters

The current study's results showed *E. coli* was the most common bacterial species isolated from patient that occurring in 50% of urinary tract infection

patients, *then Klebsiella pneumonia (20.00%), followed by Staphylococcus aureus(11.67%), Enterococcus faecalis (3(5.00%)), Pseudomonas aeruginosa(3(5.00%)), Proteus mirabilis 3(5.00%), then Staphylococcus haemolyticus, Staphylococcus hominins, Staphylococcus saprophyticus and Acinetobacter baumannii (1(1.67%)) for each isolale.*

The results showed that there was a significant difference in serum levels of FGF 23, CFH,suPAR, and another parameter at (P-value <0.05) when comparisons across patient with control groups. The concentration of all immunological markers was high in patients with renal failure with UTI and without UTI compared to the UTI and control groups. A patient with renal failure had elevated levels of urea, creatinine, phosphorus, Ferritin, and PTH.

There was a positive correlation between FGF23 and CFH ($r = 0.768$, $p < 0.001$) as well as between FGF23 and suPAR ($r = 0.674$, $p < 0.001$). CFH and suPAR have a robust correlation ($r = 0.670$, $p < 0.001$) in addition, positive correlation between FGF-23 and serum phosphorus levels ($r = 0.342$, $p = 0.008$).

It can be concluded from the current study that females are more susceptible to infection with UTI than males, and E. coli was the most common causative agent in UTI. The findings highlight the importance of monitoring FGF23, CFH, and suPAR levels in patients with renal impairment. Early detection of elevated levels can facilitate timely interventions to mitigate progression and manage complications. Understanding demographic predispositions to UTIs can inform targeted prevention strategies, particularly among younger females, to reduce infection rates and associated renal complications.

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List of abbreviations

Code	Word
AKI	Acute kidney injury
AMD	Age-related macular degeneration
ARF	Acute renal failure
AUC	Area under the curve
BAB	Blood agar base
BHI	Brain heart infusion
BP	Blood pressure
BPV	Blood pressure variability
C3G	C3 Glomerulopathy
CFH	Complement factor H
CFU	Colony-forming units
CKD	Chronic kidney disease
CKD-MBD	Chronic Kidney Disease-Mineral and Bone Disorder
CVC	Central venous catheter
CVD	Cardiovascular diseases
EIA	Enzyme immunoassay
ELISA	Enzyme-Linked Immunosorbent Assay
ESKD	End-stage kidney disease
ESRD	End-stage renal disease
ESRF	End-stage renal failure
ESRF	end-stage renal failure
FGF 23	Fibroblast growth factor 23
FHRs	Factor H–related proteins
GFR	Glomerular filtration rate
GI	Gastrointestinal
GPI	Glycosylphosphatidylinositol
HD	Hemodialysis
HTN	Hypertension
HUS	Hemolytic uremic syndrome
IgAN	IgA nephropathy
IgE	Immunoglobulin E
KRT	kidney replacement therapy
LMIC	low- and middle-income countries
MBL	mannose-binding lectin

MPGN	Membrane proliferative glomerulonephritis
mRNA	Messenger RNA
MRSA	Methicillin-resistant Staphylococcus aureus
MSA	Mannitol Salt Agar
NO.	Number
NPV	Negative predictive value
OD	Optical density
PPV	Positive predictive value
ROS	Reactive Oxygen Species
ROC	Receiver Operating Characteristic
RRT	Renal replacement therapy
RUTI	Recurrent Urinary tract infection
SD	Standerd deviation
suPAR	Soluble urokinase plasminogen activator receptor
Th2	T-helper2
UPA	Urokinase-type plasminogen activator
UPEC	Uropathogenic *Escherichia coli*
UIBC	Unsaturated Iron Binding Capacity
UTIs	Urinary tract infections

Chapter One
Introduction and Literature Review

1.1 Introduction

The kidney is an essential organ responsible for blood filtration, waste removal—including drug elimination—fluid and electrolyte balance maintenance, hormone secretion for blood pressure regulation, stimulation of red blood cell production, activation of vitamin D for calcium synthesis, and overall bone health preservation. Acute renal failure and chronic kidney disease are the primary conditions associated with renal failure (Poranki *et al*, 2020).

Chronic Kidney Disease (CKD) is defined by a progressive decline in kidney structure or function persisting for over three months, resulting in considerable health consequences. The glomerular filtration rate (GFR) is known as the most comprehensive indicator of renal function and is commonly utilized in the diagnosis, classification, and treatment of chronic kidney disease (CKD). The Kidney Disease Improving Global Outcomes (KDIGO) classification system categorises chronic kidney disease (CKD) into five stages according to glomerular filtration rate (GFR) values, where elevated stages signify diminished GFR and heightened renal impairment. In the fifth stage, the patient will advance to end-stage renal disease (ESRD) and get renal replacement therapy (RRT) (Thi and Nguyen, 2020).Renal failure refers to a condition where the kidneys lose their normal functionality. Dialysis is one of the most common strategies of renal replacement therapy.(Gattia, *et al*, 2014)

Hemodialysis increased quality of life and reduced morbidity and mortality in patients with end-stage renal illness. At the same time, contemporary society was increasingly concerned about the life experience and needs of patients throughout the treatment phase (Yujie *et al* , 2023).

The immune system and the kidneys are closely linked. Immune components facilitate acute renal failure and are essential to the advancement of chronic kidney disease. In addition to its pathogenic roles, the immune system maintains immunological homeostasis in healthy kidneys. The kidneys contribute to immunological balance by eliminating metabolic waste and toxins, therefore reducing local and systemic inflammation (Foresto-Neto *et al.*, 2024) Immune disorders, involving both innate and adaptive responses, are common in patients with end-stage renal disease under chronic hemodialysis (Angeletti et al., 2020).

FGF 23 is a bone-derived hormone that is essential for regulating vitamin D and phosphate homeostasis (Czaya and Faul, 2019). Fibroblast growth factor-23 (FGF-23) concentrations begin to increase early in individuals with chronic renal disease (Czaya and Faul, 2019). Fibroblast growth factor 23 concentrations rise in chronic kidney disease as a suitable physiological response to sustain neutral phosphate balance and normal serum phosphate levels amid diminished renal capacity for phosphate excretion. Preventing serum phosphate elevations is likely beneficial in chronic kidney disease, as studies indicate an increased risk of detrimental renal and cardiovascular outcomes linked to overt hyperphosphatemia and even slight elevations in serum phosphate within the normal range (Isakova *et al.*, 2011)(Erbak Yılmaz *et al.*, 2023)

suPAR, a soluble form of the urokinase-type plasminogen activator receptor, is a biomarker synthesised by macrophages, monocytes, neutrophils, activated T cells, endothelial cells, and circulating tumors cells. suPAR is a resulting biomarker indicative of modified inflammation in various inflammatory diseases (Erbak Yılmaz *et al.*, 2023) suPAR levels are increased in patients with CKD as compared to subjects with normal renal function (Yadav *et al.*, 2023) .

Factor H is an essential regulator of the alternative complement system, and genetic or acquired deficiencies in Factor H are linked to glomerular damage. The human Factor H–related proteins (FHRs) consist of a fifth of proteins that share structural similarities with Factor H (Renner *et al.*, 2022) .Factor H (FH) is a critical regulator of the alternative complement pathway and its deficiency or mutation underlie kidney diseases such as dense deposit disease (Mahajan *et al.*, 2021).

Infections are today the predominant problems impacting patients with chronic kidney disease, especially those receiving continuous hemodialysis (Leone and Suter, 2010). Patients receiving haemodialysis suffer an increased risk of severe infection and mortality due to various factors (Sannathimmappa *et al.*, 2024).

So the present study aimed to :

1- Isolation and identification bacterial pathogens of urinary tract infection under frequent hemodialysis.
2- Evaluation the concentration of immunological markers (soluble urokinase plasminogen activator receptor suPAR, complement factor H CFH, fibroblast growth factor 23 FGF23) in patients with UTI not on hemodialysis, hemodialysis patients with and without UTI, compared with healthy controls.
3- Detection the association between the serum levels of fibroblast Growth Factor 23 (FGF23), Soluble Urokinase Plasminogen Activator Receptor, and Complement Factor H (CFH) among patients with chronic kidney failure disease and a study the correlation among these biomarker in patients and control groups.

4- Determintion and evaluatintion the level of immunity and immune markers (FGF 23,suPAR,and CFH) based on kidney functions, and some other parameters.
5- Correlation between FGF 23 with biochemical, hematological, and parathyroid hormone parameters in renal failure patients.

1.2 Literature Review :

1.2.1. Overview of Renal Failure

Kidney disease is defined as a heterogeneous group of disorders affecting kidney structure and function. It is recognized now that even mild abnormalities in measures of kidney structure and function are associated with increased risk for developing complications in other organ systems as well as mortality, all of which occur far more frequently than kidney failure (Levey, *et al*, 2013) .

Renal failure has numerous potential etiologies. Certain factors result in a rapid decline of renal function (acute kidney damage, also referred to as acute renal failure). Another result in a progressive deterioration of renal function is chronic kidney disease, also known as chronic renal failure. In addition the kidneys' inability to filter metabolic waste products (such as creatinine and urea nitrogen) from the bloodstream, they also exhibit diminished capacity to regulate fluid balance and maintain appropriate levels of electrolytes (sodium, potassium, calcium, phosphate) and acid in the blood (Malkina, 2023).

Kidney disease is characterized by the impairment of renal function and causes accumulation of blood metabolites, which alter the electrolyte balance (Calvo-Lobo *et al.*, 2019) .

1.2.2. Causes of renal failure :

There are several factors that may lead to the progress of acute renal failure conditions such as hypotension, a blockage of the urinary tract, hemolytic uremic syndrome and some medications. Diabetes, hypertension, polycystic renal disease and nephrotic syndrome represent the major causes of chronic renal failure (Mohsen, *et al*, 2023) .

The number of CKD patients will continue to rise, reflecting the growing elderly population and increasing numbers of patients with diabetes and hypertension (Alkhaqani, 2022) .

1.2.2.1. Diabetic Mellitus

It is one of the most common causes of chronic kidney disease (CKD) and end-stage renal disease (ESRD), Persistent high blood glucose levels can damage the small blood vessels in the kidneys, impairing their ability to filter waste and fluids from the blood effectively.(Jha *et al.*, 2024). Diabetes is the predominant etiology of chronic kidney disease (CKD) and end-stage renal disease (ESRD) globally. 20 to 30% of diabetic patients exhibit diabetic nephropathy in both type 1 and type 2 diabetes (Shahbazian and Rezaii, 2013).

Diabetic kidney disease (DKD) constitutes the leading cause of end-stage kidney disease and occurs as a result of renal microvascular lesions, causing distinct and progressive morphological changes, inducing albuminuria and progressive loss of kidney function.(Poloni and Rotta, 2022). It affects up to 50% of individuals with diabetes, serves as a primary contributor to end-stage kidney disease (ESKD) necessitating dialysis or renal transplantation, and is linked to markedly elevated cardiovascular morbidity and death (Selby and Taal, 2020).

The primary indicator of DKD is albuminuria, which correlates with the advancement of renal disease and cardiovascular incidents (Lin *et al.*, 2018). Diabetic nephropathy is the predominant cause of end-stage renal failure (ESRF) globally, accounting for nearly 50% of those undergoing renal replacement treatment in certain regions. The condition is prevalent in individuals with type 1 and type 2 diabetes, although its occurrence seems to be decreasing, particularly in type 1 diabetes. Over one-third of individuals with type 2 diabetes exhibit compromised renal function. Progress in our comprehension of the pathophysiology and natural history of the illness has allowed us to contemplate earlier interventions focused on renal preservation and the mitigation of cardiovascular morbidity. Microalbuminuria is recognized as the initial risk indicator for nephropathy in type 1 diabetes and cardiovascular disease in type 2 diabetes (Eboh and Chowdhury, 2015).

Chronic hyperglycemia is the principal factor in macrovascular and microvascular problems linked to diabetes mellitus. Excess hyperglycemia induces redox imbalance and both systemic and intrarenal inflammation, significantly contributing to the pathophysiology of diabetic kidney disease, which is the primary cause of dialysis globally. The pathophysiology of the disease is intricate, multifaceted, and not entirely understood; numerous causes and mechanisms contribute to its development, progression, and clinical results. Although the mechanisms behind kidney impairment associated with diabetes mellitus are varied, the metabolic pathways involving oxidative and inflammatory processes are broadly acknowledged. There is unequivocal evidence that a persistent hyperglycemic state induces oxidative stress and inflammation through modified metabolic pathways in a self-sustaining cycle, facilitating the advancement of cellular damage and end-stage renal disease(Amorim *et al.*, 2019)

1.2.2.2. Hypertension

Hypertension is prevalent in adults with chronic kidney disease (CKD), affecting 65% to 85% of patients and exacerbating as renal function deteriorates. The pathogenesis of hypertension in chronic kidney disease is intricate, mostly associated with diminished nephron mass, overactivation of the sympathetic nervous system, participation of the renin-angiotensin-aldosterone system, and widespread endothelial dysfunction. Consensus guidelines for blood pressure targets recommend a blood pressure of <120/80 mm Hg for those with native chronic renal disease and <130/80 mm Hg for kidney transplant patients. Guidelines highly recommend renin-angiotensin-aldosterone system blocking as the primary treatment (Hebert and Ibrahim, 2022)

Hypertension is frequently inadequately managed in individuals with chronic kidney disease (CKD). Precise blood pressure (BP) assessment is the fundamental initial step in the diagnosis and management of hypertension(Georgianos and Agarwal, 2023). Uncontrolled hypertension can result in considerable cardiovascular morbidity and mortality, as well as hasten the progression to end-stage renal disease(Ku *et al.*, 2019)

Hypertension continues to be one of the most detrimental consequences of chronic kidney disease (CKD). It is believed to expedite the gradual deterioration of renal function, cardiovascular diseases (CVD), and associated mortality. The detection and management of hypertension are often inadequate, and enhancements could directly benefit patients. The Systolic Blood Pressure Intervention Trial yielded significant insights on the impact of aggressively reducing systolic blood pressure to a target of <120 mm Hg, which may be pertinent to CKD patients; however, this trial excluded high-risk individuals with CKD, proteinuria, or diabetes. Modifications in lifestyle, including weight

reduction and dietary sodium limitation, may enhance blood pressure regulation. Such interventions may be less expensive than pharmacological medicines and possess the capacity to influence outcomes, such as heart failure and stroke, in both advanced healthcare systems and low- and middle-income countries (LMICs), a viable objective would be to enhance the management of hypertension-related problems in chronic kidney disease patients, with the aim of reaching target blood pressure ranges for a significant number of individuals. This objective can be achieved worldwide, and its effects are readily quantifiable(Alkhaqani, 2022)

1.2.2.3. Bacterial infections

Bacterial infections are a significant cause of renal failure, potentially resulting in both acute and chronic kidney diseases. The relationship between bacterial infections and renal failure is complex, encompassing direct bacterial invasion, immune-mediated injury, and secondary consequences arising from systemic infections(Hassooni *et al.*, 2018). Bacterial infections may result in glomerulonephritis, an inflammation of the kidney's filtration units, potentially leading to acute renal failure. This syndrome may result from direct bacterial invasion or the deposition of immune complexes subsequent to infections such as poststreptococcal glomerulonephritis(Zeledon *et al.*, 2008). Bacterial infections induce kidney damage by direct infection of the renal parenchyma, immune-mediated damage, and blockage resulting from infection-related implications(Williams, Bhagani and Harber, 2014).

Obstructive uropathy is an important cause of acute and chronic kidney disease. Decompression of the urinary tract is an essential aspect of treatment. The cause and aetiology of obstruction typically determine the surgical approach. Acute relief of obstruction is frequently complicated by fluid and electrolyte imbalance.

Standard therapeutic interventions for acute or chronic renal failure also apply for cases of obstructive uropathy(Yaxley and Yaxley, 2023).

Obstruction of the urinary tract can occur at any point from the calyces to the external urethral meatus. Urinary tract obstruction is best divided into upper tract and lower tract obstruction. Obstruction can be acute or chronic. Acute upper tract obstruction is most commonly due to a calculus and acute lower tract obstruction in men is often due to benign prostatic enlargement.(Hall and Linton, 2008) Obstructive uropathy is a significant cause of chronic kidney disease (CKD), leading to hemodialysis treatment, urinary tract stones were the most common cause of obstructive uropathy among patients undergoing hemodialysis (Sembiring, Daryanto and Gunawan, 2024).

1.2.3. Acute Renal Failure

Acute kidney damage, previously referred to as acute renal failure, is a syndrome defined by the swift decline of the kidney's excretory function, often identified by the accumulation of nitrogenous waste products (urea and creatinine) or reduced urine production, or both. It is the clinical presentation of several illnesses that acutely impact the kidneys (Bellomo, Kellum and Ronco, 2012). Acute renal failure (ARF) is a phenomenon defined by a rapid decrease in glomerular filtration rate within hours or days, resulting in the kidneys' inability to excrete nitrogenous waste and sustain homeostasis (Diallo *et al.*, 2024).

Acute renal failure (ARF) is a common issue in the critical care unit and is linked to elevated death rates (Trof *et al.*, 2006). Acute Kidney Injury (AKI) is linked to a heightened risk of Chronic Kidney Disease (CKD) development, progression, and mortality (Siew *et al.*, 2012).

Acute kidney injury (AKI) in hospital settings is prevalent and correlates with increased mortality rates (Go *et al.*, 2018) Acute kidney damage may elevate the risk of chronic kidney disease and end-stage renal disease.The correlation between AKI and CKD or ESRD was assessed according to the severity of AKI, and the effect magnitude was mitigated by a reduced baseline glomerular filtration rate.Acute kidney injury (AKI) is linked to elevated morbidity and mortality rates in both the short and long term. Survivors face an elevated risk of chronic kidney disease and end-stage renal disease (Pota and Bell, 2024). Though creatinine levels in the majority of patients with acute kidney injury (AKI) typically normalize with time, numerous studies indicate that AKI increases the risk of death, cardiovascular incidents, and chronic kidney disease (CKD)(Wenwen Zhang *et al.*, 2024).

The classification of acute kidney injury (AKI) encompasses pre-renal AKI, acute post-renal obstructive nephropathy, and intrinsic acute kidney disorders. Among these, only 'intrinsic' AKI constitutes genuine renal pathology, whereas pre-renal and post-renal AKI result from extrarenal conditions that diminish the glomerular filtration rate (GFR). If these pre- and/or post-renal circumstances continue, they will ultimately lead to renal cellular damage and, consequently, intrinsic renal disease (Makris and Spanou, 2016). Renal impairment Lasting between 7 and 90 days following an acute kidney injury (AKI) is referred to as acute kidney disease (AKD) (Chen *et al.*, 2020). The advancement of chronic kidney disease may transpire through mechanisms unrelated to the initial acute pathological condition or injury. Cascading mechanisms related to progressive injury encompass systemic and intrarenal hypertension, glomerular hyperfiltration, tubular hypertrophy and atrophy, tubulointerstitial fibrosis, progressive glomerular sclerosis, arteriosclerosis, genetic predisposition, and dysregulated humoral responses linked to chronic kidney disease (Chawla *et al.*, 2014).The

Pathophysiological Features of Acute Kidney Injury Leading to Chronic Kidney Disease are illustrated in figure 1.1

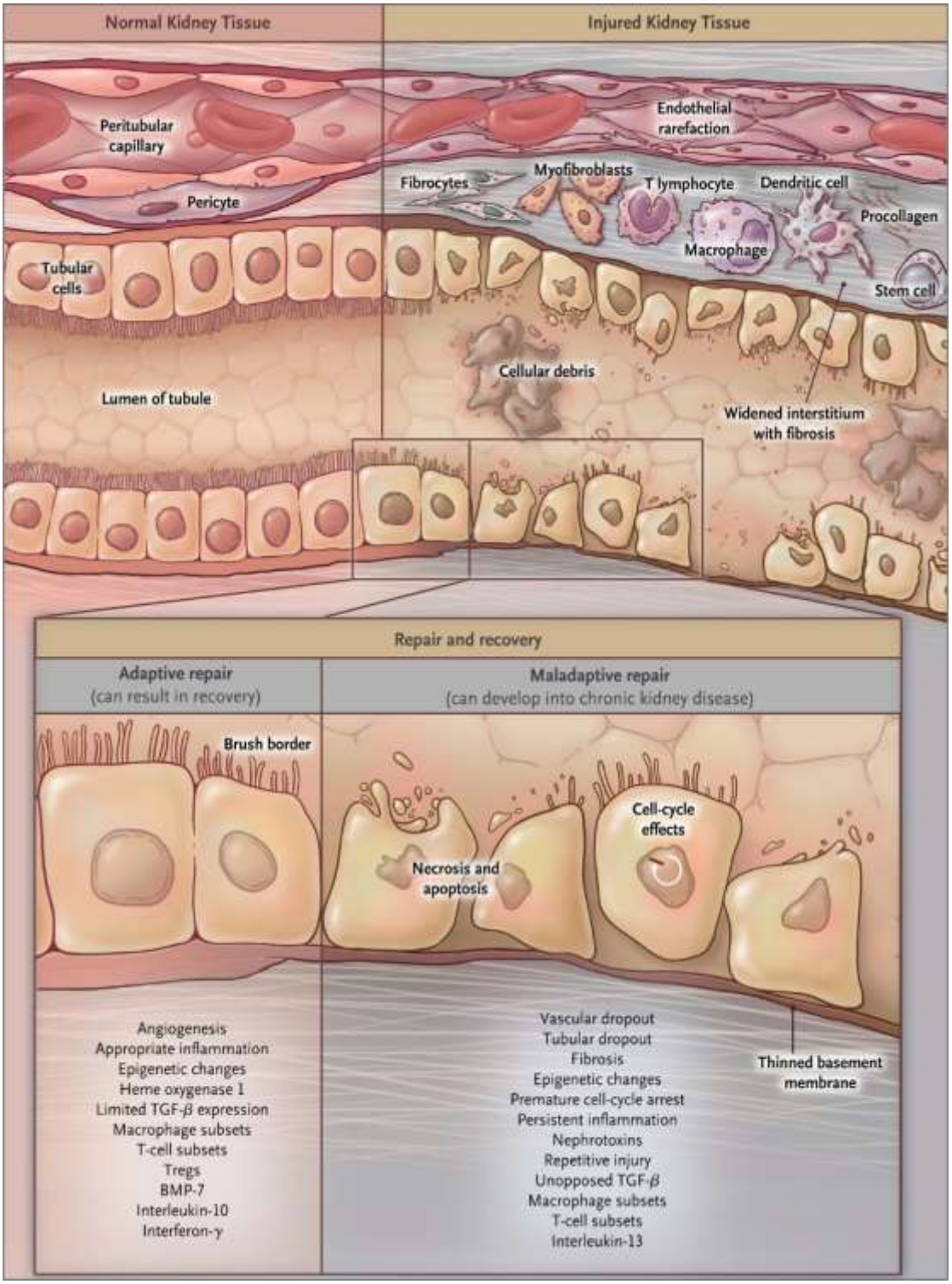

Figure 1-1: Pathophysiological Features of Acute Kidney Injury Leading to Chronic Kidney Disease.(Chawla *et al.*, 2014)

1.2.4. Chronic kidney disease

Kidney disease is defined by a decline in renal function, resulting in the accumulation of blood metabolites that disrupt electrolyte balance(Calvo-Lobo, Neyra-Bohorquez and Seco-Calvo, 2019). Recent studies identified a genetic cause in 10% to 20% of adults with CKD.(Lamarche, Iliuta and Kitzler, 2019)

Chronic kidney disease (CKD) constitutes a global public health issue, characterized by increasing prevalence, expenses, and adverse outcomes. Individuals with chronic renal insufficiency are susceptible to numerous problems. Immunological dysfunction is a significant and severe consequence in these people (Bondar, Klimontov and Simakova, 2011)

Chronic kidney disease is one of the leading causes of death worldwide today.(Sahin and Yıldıran, 2024) Chronic Kidney Disease (CKD) is characterized by kidney damage or a glomerular filtration rate (GFR) of ≤60 mL/min/1.73m² persisting for over three months. Chronic Kidney Disease (CKD) is categorized into five stages according to a decrease in Glomerular Filtration Rate (GFR)(Shen *et al.*, 2019)

Chronic kidney disease (CKD) and its progression to end-stage renal disease (ESRD) constitute a developing global health problem, with hypertension and diabetes frequently recognized as principal etiological contributors (Alhazmi *et al.*, 2023) Early chronic kidney disease is asymptomatic, with symptoms emerging only in later stages when problems emerge, including diminished kidney function and the existence of related comorbidities. In the advanced phases of the disease, when renal function is markedly compromised, patients can alone be managed

through dialysis or transplantation. Due to restricted treatment alternatives, a growing senior demographic, and the rise of comorbidities linked to the disease, the incidence of chronic kidney disease (CKD) is anticipated to increase. This review addresses the prevailing obstacles and the unfulfilled patient requirements in chronic kidney disease (CKD) (Evans *et al.*, 2022)

Early detection is an essential strategy for preventing kidney disease, its progression, and associated problems; yet, multiple studies indicate that public knowledge of renal illness is insufficient. Consequently, enhancing awareness and executing sustainable strategies for the early identification of kidney illness are crucial public health objectives. Economic and epidemiological data highlight the necessity of prioritizing kidney disease on the global public health agenda, since its incidence is rising globally and it has become the seventh highest risk factor for mortality worldwide. Furthermore, demographic trends, the obesity epidemic, and the consequences of climate change are expected to exacerbate the prevalence of kidney disease, significantly impacting survival, quality of life, and medical costs. Global (Francis *et al.*, 2024).

The estimated prevalence of chronic kidney disease (CKD) is 10% of the global population, however it exhibits significant variability worldwide. In total, 850 million individuals are impacted by varying degrees of chronic kidney disease (CKD), with 85% residing in low- to middle-income nations. The primary risk factors for chronic kidney disease are age, hypertension, diabetes, obesity, proteinuria, dyslipidemia, and environmental variables such as dietary salt consumption and a recently studied agent (Torino *et al.*, 2018) Kidney failure, the final stage of chronic kidney disease (CKD), constitutes a significant health issue wherein the kidneys stop to function adequately. Owing to the rising incidence of type 2 diabetes, hypertension, and obesity, it is anticipated that by 2030, there will

be a significant increase in those at risk for this lethal disease globally (Raoofi *et al.*, 2023). The mortality rate among patients on hemodialysis is significantly elevated and correlates with non-modifiable characteristics like age, as well as modifiable factors such as the type of vascular access and nutritional state at the onset of therapy (de Arriba *et al.*, 2021).

The survival rate of hemodialysis patients decreased around the five-year mark. Negative prognostic factors present at the start of hemodialysis include diabetes, hypertension, and chronic anemia; additional factors that emerge afterwards encompass malnutrition, hypoalbuminemia, cardiovascular disease, and liver disease (Valdivia *et al.*, 2013) The global increase in obesity and diabetes has elevated the incidence of chronic kidney disease (CKD) (Aljawadi *et al.*, 2024).

1.2.5. End-stage renal failure

End-stage renal disease (ESRD) affects over 1500 individuals per million population in areas with a high prevalence(Abbasi, Chertow and Hall, 2010) in the past two decades, There has been growing recognition of an excess of end-stage renal disease (ESRD) cases necessitating renal replacement therapy, lacking common underlying causes such as diabetes, hypertension, glomerulonephritis, or any identifiable etiology, in numerous low-to-middle income countries. End-stage renal disease of unknown cause predominantly impacts young adults of working age and is a significant worldwide health issue characterized by considerable morbidity, mortality, and disability (Fiseha and Osborne, 2023)

The global epidemiology of end-stage kidney disease (ESKD) shows the distinct genetic, environmental, lifestyle, and sociodemographic attributes of every country. The approach to end-stage kidney disease (ESKD), especially concerning kidney replacement treatment (KRT), is influenced by local disease prevalence,

cultural factors, and socioeconomic conditions (Thurlow *et al.*, 2021). End-stage renal disease (ESRD) is identified when renal function is insufficient for prolonged survival without kidney transplantation or dialysis (Wouk, 2021).

Hemodialysis (HD) is a life-saving treatment for patients with end stage renal disease.(Poppelaars *et al.*, 2018) For the majority of individuals with end-stage renal failure, dialysis can prolong their lifespan. Nonetheless, treatment may be difficult and time-intensive. Throughout dialysis treatment, numerous people persist in enduring diverse afflictions(Beng *et al.*, 2019). Symptoms of renal failure include vomiting, edema in the lower extremities, anorexia, disorientation, and fatigue. Multiple problems arose, including hyperkalemia, volume overload, and uremia in the acute phase, as well as hypertension, anemia, and cardiovascular disease in the chronic phase (Mohsen, Maaroof and Alduhaidhawi, 2023).

Hypertension and diabetes mellitus are the primary etiological factors for end-stage renal failure in those over 40 years of age(Banaga *et al.*, 2015). Chronic pain that is prevalent in patients with end-stage renal disease (ESRD), is generally moderate to severe, and affects nearly all dimensions of health-related quality of life. However, there is insufficient clinical and research interest in nephrology regarding this area, resulting in inadequate treatment of pain in end-stage renal disease (ESRD) (Davison, 2005) End-stage renal disease (ESRD) causes premature ageing of the immune system(Ducloux *et al.*, 2018) Chronic inflammation is a fundamental characteristic of end-stage renal disease and correlates with heightened mortality and morbidity risk in patients receiving dialysis.

1.2.6. Hemodialysis:

1.2.6.1. Definition

The term dialysis originates from the Greek words dia, meaning "through," and lysis, meaning "loosening or splitting." This is a type of renal replacement therapy in which the kidney's function of blood filtration is augmented by artificial devices that eliminate excess water, solutes, and poisons. Dialysis facilitates the preservation of homeostasis in individuals undergoing a swift decline in renal function, either acute kidney injury (AKI), or the gradual, progressive deterioration characteristic of chronic kidney disease (CKD)(Murdeshwar and Anjum, 2024).

Haemodialysis is the predominant technique employed to eliminate waste and toxic substances from the body, hence serving as a treatment for individuals with various forms of renal failure(Mehmood *et al.*, 2019).

Patients undergoing hemodialysis (HD) are susceptible to several early and long-term complications, including chronic inflammation, infections, malnutrition, and cardiovascular disease, which considerably influence mortality rates (Losappio *et al.*, 2020).

Although hemodialysis is a primary therapeutic modality for chronic kidney failure, it also induces numerous health complications. A range of alternative and integrative therapies is employed to alleviate or reduce hemodialysis symptoms. Fatigue (60%-97%) is among these problems (Bayülgen and Gün, 2022)

Hemodialysis (HD) is recognised for inducing a chronic inflammatory condition that impacts both the innate and adaptive immune responses(Donadei *et al.*, 2023) Immune disorders, affecting both innate and adaptive responses, are prevalent in individuals with end-stage renal disease undergoing chronic hemodialysis (Angeletti *et al.*, 2020). Patients undergoing hemodialysis (HD)

exhibit heightened risk to infections, particularly from resistant pathogens (Shamas *et al.*, 2024).

1.2.6.2. History of hemodialysis

Development of dialysis by pioneers like Willem Kolff and Belding Scribner initiated major changes in the epidemiology, economics, and ethical considerations around kidney failure therapy (Himmelfarb *et al.*, 2020) In 1943, Kolff administered treatment to his first patient using an artificial kidney—a young woman who underwent effective dialysis 12 times but ultimately succumbed to vascular access failure (Peitzman, 2001).

Haemodialysis (HD) is the predominant modality of kidney replacement therapy globally, constituting over 69% of all kidney replacement therapies and 89% of all dialysis procedures (Bello *et al.*, 2022). Ten percent of the global population is afflicted by chronic kidney disorders, with 2.6 million individuals currently receiving hemodialysis (HD),projected to rise to approximately 5.4 million by 2030 (Campo *et al.*, 2022).

The first two weeks of chronic dialysis correlate with increased mortality and hospitalization risks, which persist elevated throughout the subsequent 90 days (Chan *et al.*, 2011). Long-term hemodialysis (HD) is the primary method employed in the management of end-stage renal disease (ESRD) (Habas *et al.*, 2021).

The global epidemiology of end-stage kidney disease (ESKD) shows the distinct genetic, environmental, lifestyle, and sociodemographic attributes of each nation. The reaction to end-stage kidney disease (ESKD), especially concerning

kidney replacement treatment (KRT), is influenced by local disease prevalence, cultural factors, and socioeconomic conditions. .(Thurlow *et al.*, 2021).

1.2.6.3. The mechanism of Hemodialysis

The dialysis process uses the concentration gradient across the membrane (dialyzer) for separation. The method involves two primary streams on opposite sides of the membrane: one with a higher concentration of targeted chemicals and the other with zero or lower concentrations (dialyzing fluid or dialysate) (Mollahosseini, Abdelrasoul and Shoker, 2020).

Dialysis depends on the migration of solute particles over a semipermeable membrane (diffusion). Urea and creatinine diffuse down the concentration gradient from the bloodstream into the dialysate, which consists of sodium bicarbonate, sodium chloride, acid concentrate, and deionized water. The dimensions of particles affect the rate of their diffusion into the dialysate, chevalier. The diffusion rate across the membrane diminishes as the solute particle size increases(Rolo, 2022).

Hemodialysis (the diffusion of molecules across a membrane) and hemofiltration (the concurrent transport of molecules and solvent across a membrane) effectively eliminate both small and medium-sized solutes from the blood into the dialysate fluid (Thajudeen, Issa and Roy-Chaudhury, 2023).

In figure (1.2), two microscopic needles will be inserted into arteriovenous (AV) fistula or graft and secured in place. Blood is gradually withdrawn through one needle and directed to a dialyzer or dialysis machine, which consists of numerous membranes functioning as filters and a specific fluid known as dialysate (Coemans *et al.*, 2018).

Membranes filter waste from the blood, which is then conveyed to the dialysate fluid. Following the expulsion of the utilized dialysate fluid from the dialyzer, the purified blood is reintroduced into the body via a second needle. Hemodialysis is not inherently unpleasant; however, patients may experience nausea, dizziness, or muscle cramping during the procedure. Blood fluid levels rapidly vary during the process; following dialysis therapy, the needles are extracted, and hemostasis is achieved with a plaster (Coemans *et al.*, 2018). . Figure (1.2) the hemodialysis schematic diagram .

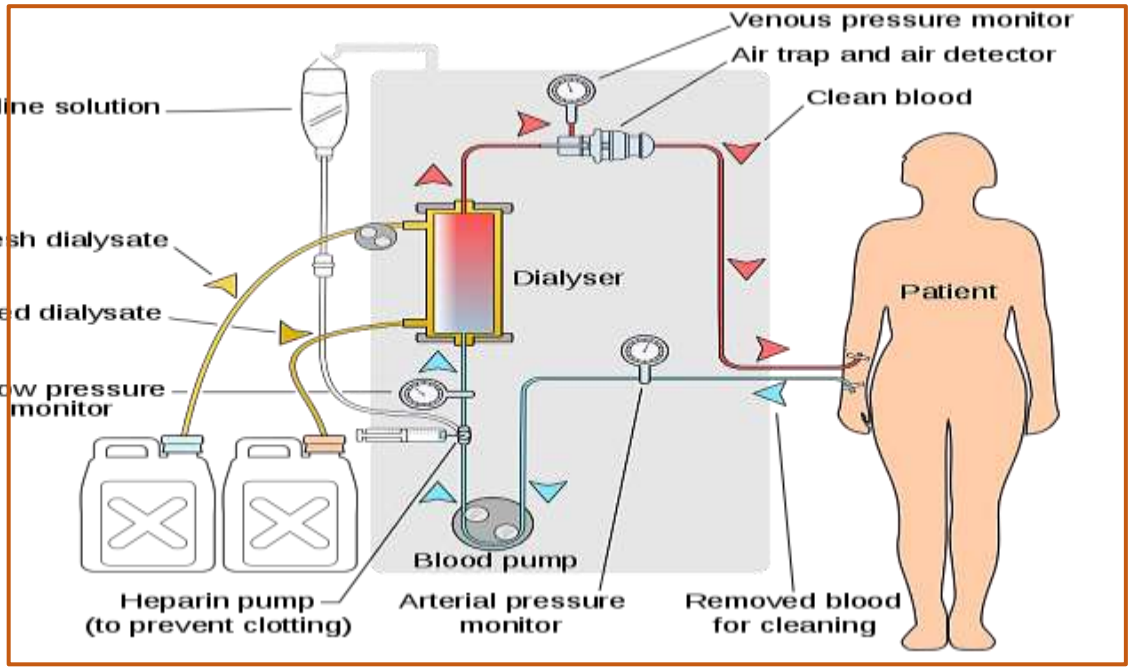

Figure (1.2) the hemodialysis schematic diagram (Coemans et al. 2018).

1.2.7. Immunity and hemodialysis

The immune system and the kidneys are intricately interconnected. Immune components promote acute renal disease and are essential to the development of chronic kidney disease. In addition to its pathogenic roles, the immune system maintains immunological homeostasis in healthy kidneys. The kidneys maintain

immunological balance by eliminating metabolic waste and toxins, therefore they are reducing local and systemic inflammation (Foresto-Neto *et al.*, 2024)

Patients undergoing hemodialysis exhibit dysregulated immune responses (González-Cuadrado *et al.*, 2023). End Stage Renal Disease (ESRD) is characterised by abnormalities in both the innate and adaptive immune systems, resulting in a simultaneous presence of immunological activation and immune repression (Sharif *et al.*, 2015). Both innate and adaptive immunity contribute to immune system senescence, renal fibrosis, and the ageing process (Sepe *et al.*, 2022).

End-stage renal disease (ESRD) is characterized by significant dysfunctions in both the innate and adaptive immune systems, indicating a state of dysregulated deactivation and immunosuppression (Franzin *et al.*, 2023).

1.2.7.1. Innate immunity

The innate immune system is a crucial regulator of the inflammatory response during infection and tissue injury or repair. The kidney, as a crucial organ with significant energy requirements, is vital in controlling disease-related metabolic processes (Wang and Zhang, 2017).

Renal inflammation stimulates and activates immune cells, significantly contributing to tissue scarring in the affected kidney. Studies have indicated a significant involvement of innate immunity in the pathological foundation of renal disorders (Tang *et al.*, 2020).

Patients undergoing hemodialysis (HD) show a different innate cell profile in comparison to healthy individuals (Valentini *et al.*, 2022).

1.2.7.2. Adaptive immunity

Immunological dysregulation, encompassing both innate and adaptive responses, is important during hemodialysis sessions and in chronic maintenance therapies. A substantial activation of the complement system is facilitated by the surfaces of dialysis membranes. These effectors create a lasting systemic pro-inflammatory, and pro-coagulant environment known as inflammation. The adaptive response, the imbalance in the CD4+/CD8+ T cell ratio, the decrease in Th2 and regulatory T cells, and the modified interaction with B lymphocytes via CD40/CD40L are mostly associated with immune system failure. Collectively, these data indicate that interventions aimed at the immune system in HD patients may enhance morbidity and mortality outcomes (Losappio *et al.*, 2020)

1.2.7.3. Key Immunological Markers in Hemodialysis:

Renal failure Patients have immunological dysregulation induced by Hemodialysis (HD) operations. Interaction between the dialysis membrane and blood will elicit bio-incompatibility responses, resulting in complement activation and the generation of Reactive Oxygen Species (ROS) with pro-inflammatory cytokines. Imbalances in the immune response will result in an immunocompromised state (Puspitawati, P and Suromo, 2018)

1.2.7.3.1. Complement factor H

The complement system is an essential part of the innate immune system and enhances adaptive immunological responses. Complement activation is recognised to occur in various renal pathologies, including glomerulonephritis, thrombotic microangiopathies, and transplant rejection (Fearn and Sheerin, 2015)

The complement system is integral to the innate immune response and also contributes to the pathophysiology of several diseases. Elements of the complement system, such as mannose-binding lectin and C3, are linked to cardiovascular disease risk in individuals with end-stage kidney disease (ESKD) (Skinner *et al.*, 2021).

Factor H is the main regulatory protein of the alternative pathway of complement activation. Abnormalities in factor H have been linked to renal pathology, namely glomerulonephritis characterised by C3 deposition, including membranoproliferative glomerulonephritis (MPGN) and atypical haemolytic uraemic syndrome (aHUS) (Pickering and Cook, 2008). The 155-kDa glycoprotein, complement factor H (CFH), serves as a regulator of complement activation and is prevalent in human plasma (Pickering and Cook, 2008).

Factor H (FH) is the principal regulator of the main complement protein C3b in the alternative pathway of complement activation and consists of 20 SCR domains. (Perkins *et al.*, 2010)

The complement cascade is an essential element of both the innate and adaptive immune systems. Complement activation also plays a role in the aetiology of numerous illnesses, with the kidney being especially vulnerable to complement-mediated damage. Pharmaceuticals that inhibit complement activation can swiftly diminish tissue inflammation and concurrently mitigate the adaptive immune response to exogenous and endogenous antigens (Thurman and Le Quintrec, 2016)

Complement is activated in the tubular lumen via the alternative pathway, and the products of complement activation are deposited on the apical surface of tubular epithelial cells (Tang and Sheerin, 2009) Complement activation is implicated in the pathophysiology of both renal and non-renal diseases. Although

the complement cascade is a crucial element of the innate immune system, its excessive activation can lead to severe pathology (Willows, Brown and Sheerin, 2020) .

Factor H (FH) which is an essential complement regulator, modulates complement activation in plasma and on the cellular surfaces of autologous tissues. FH has an evolutionary lineage and structural characteristics with a category of plasma proteins termed FH-Related Proteins (FHRs), which may function as antagonists to FH. Research involving patient cohorts with atypical Haemolytic Uraemic Syndrome (aHUS), C3 Glomerulopathy (C3G), and IgA nephropathy (IgAN) has discovered infrequent genetic variations that lead to significant dysfunctions in FH and FHRs, serving as primary genetic predispositions (Gómez Delgado and Sánchez-Corral, 2022) Complement factor H (CFH) is an essential inhibitory protein of the alternative pathway, functioning by competing with C3 convertase and serving as a cofactor for factor I in the cleavage of C3b (Wen *et al.*, 2019)

1.2.7.3.2 - Soluble urokinase plasminogen activator receptor

Soluble urokinase plasminogen activator receptor (suPAR) is a blood protein that indicates the severity of systemic inflammation (Griveas *et al.*, 2021)

suPAR is a ubiquitous and non-specific biomarker of inflammation, produced in response to inflammatory stimuli irrespective of the underlying aetiology. It is a soluble form of the urokinase receptor, a cell membrane receptor with a glycosylphosphatidylinositol (GPI) anchor that is selective for the urokinase-type plasminogen activator. Urokinase cleaves plasminogen into plasmin, thereby initiating the fibrinolysis cascade for the destruction of thrombi in blood vessels. suPAR is a 60-kDa glycoprotein produced from the cleavage of the

GPI anchor, believed to possess a chemotactic activity that facilitates leukocyte tissue migration. It also participates in tissue remodelling following trauma. The analysed molecule is the active variant of a membrane-bound receptor present on various cell types, including leukocytes, fibroblasts, tissue macrophages, endothelial cells, cardiomyocytes, and renal tubular cells (Velissaris *et al.*, 2021)

The soluble receptor of urokinase plasminogen activator (suPAR) serves as a biomarker for innate immunity and inflammation, forecasting cardiovascular and non-cardiovascular events across diverse situations, including type 2 diabetic patients undergoing dialysis. Nonetheless, the correlation between suPAR and clinical outcomes across the broader haemodialysis population remains unexamined (Torino *et al.*, 2018).

The soluble urokinase receptor (suPAR) is a potential biomarker linked to the incident of chronic kidney disease (CKD), with plasma suPAR levels exhibiting little change over a six-month period (Weidemann *et al.*, 2020). The biomarker soluble urokinase plasminogen activator receptor (suPAR) serves as an indication of inflammation, exhibiting elevated levels in several chronic and acute illness conditions (Velissaris *et al.*, 2022). The prognostic significance of suPAR in different renal disorders has recently been established (Jehn *et al.*, 2021).

Increased concentrations of soluble urokinase-type plasminogen activator receptor (suPAR) and additional fibrinolytic markers associated with the urokinase-type plasminogen activator (uPA) system may be involved in plasma clot lysis (Pawlak, Pawlak and Mysliwiec, 2007). Levels of soluble urokinase plasminogen activator receptor correlate with declining kidney function (Drechsler *et al.*, 2017).

Strong evidence indicates that suPAR serves as a predictive marker for adverse events, morbidity, and mortality. It is linked to immunological function and outcomes in various illnesses, including renal disease, cardiovascular disease, malignancies, diabetes, and inflammatory disorders (Rasmussen, Petersen and Eugen-Olsen, 2021). The heart and kidneys as organs are responsible for suPAR clearance in humans (Chew-Harris *et al.*, 2019).

Inflammation is a significant factor in the progression of numerous diseases. The soluble urokinase plasminogen activator receptor (suPAR) is a highly adaptable molecule possessing inherent chemotactic characteristics. This glycoprotein has been evaluated as a biomarker for inflammation, immunological activation, organ damage, and clinical outcomes across various pathologies, including cardiovascular disease, hepatitis, renal illnesses, and rheumatic conditions. The utilisation of this early warning inflammatory biomarker may enhance the prognosis of disease severity and death (Desmedt *et al.*, 2017).

Soluble urokinase plasminogen activation receptor (suPAR) is a risk factor for renal disease (Sommerer *et al.*, 2019)

1.2.7.3.3. Fibroblast growth factor 23

Human FGF23, a 32-kDa glycoprotein, is encoded by a gene comprising 9386 nucleotides located on chromosome 12p13, which contains three exons and two introns(Goetz *et al.*, 2007). It is part of the fibroblast growth factor (FGF) class. The 22 members of the FGF superfamily participate in numerous biological processes, such as development, organogenesis, and metabolism, and have been phylogenetically categorised into seven subfamilies (Itoh, Ohta and Konishi, 2015).

FGF23 is predominantly synthesised by osteocytes and osteoblasts. However, diminished amounts of FGF23 messenger RNA (mRNA) have been identified in the brain, thymus, spleen, small intestine, heart, and testis. The physiological function of FGF23 synthesised in various tissues remains unclear (Yoshiko *et al.*, 2007).

Fibroblast Growth Factor 23 (FGF-23) is a hormone originating bone that plays a crucial role in the regulation of phosphate homeostasis. FGF-23 concentrations are markedly increased in Chronic Kidney Disease (CKD), and evidence substantiates the involvement of this hormone in the pathophysiology of Chronic Kidney Disease-Mineral and Bone Disorder (CKD-MBD). Moreover, recent data links FGF-23 to the pathophysiology of systemic problems in CKD-MBD. The growing understanding that the effects of faulty mineral metabolism extend beyond bone disease has altered the approach to the pathogenesis and treatment of disrupted bone and mineral metabolism in individuals with chronic kidney disease (CKD). FGF-23 is posited as the primary adaptive response in early chronic kidney disease to safeguard the organism from the detrimental consequences of phosphate retention. Elevated FGF-23 levels in CKD patients correlate with cardiovascular mortality risk and have been demonstrated to induce direct, “off-target” damage to the heart (Diniz and Frazão, 2013).

Fibroblast growth factor 23 (FGF23) is predominantly synthesised in the bone and, upon release, associates with a FGF receptor and the coreceptor αKlotho. FGF23 can perform various endocrine roles, including the inhibition of renal phosphate reabsorption and the synthesis of 1,25-dihydroxyvitamin D3 (Bär *et al.*, 2019).

Fibroblast growth factors (FGFs) are signalling proteins that play diverse roles in cellular development, repair, and metabolism. The human FGF family has 22 structurally related members, categorised into three distinct groups according to its methods of action: intracrine, paracrine/autocrine, and endocrine FGF subfamilies. FGF19, FGF21, and FGF23 are members of the hormone-like/endocrine FGF subfamily. These endocrine FGFs primarily regulate cellular metabolic functions, including the equilibrium of lipids, glucose, energy, bile acids, and minerals (phosphate/active vitamin D). Endocrine FGFs operate via a distinct protein family known as klotho. Two family members, α-klotho and β-klotho, serve as principal cofactors that can scaffold to bind FGF19/21/23 to their respective receptors (FGFRs) to create an active complex (Zimmerman, 2022).

FGF-23, known as phosphatonin, is a hormone that modulates renal phosphorus excretion and has been identified as an early biomarker for acute kidney injury (AKI) and the prognosis of negative outcomes in patients with confirmed AKI. FGF-23 is markedly elevated in the urine and blood of patients with acute kidney injury (AKI) and in many experimental models, including ischemia/renal reperfusion, folic acid-induced AKI, and rhabdomyolysis. Clinical evidence demonstrates that circulation levels of FGF-23 markedly elevate in newborns, children, adults, and the elderly with acute kidney injury (AKI) . The decreased clearance of FGF-23 in individuals with acute kidney injury (AKI) further elevates circulating levels of FGF-23. Numerous studies have elucidated the mechanisms responsible for the elevation of FGF-23 in acute kidney injury (AKI), with its quantitative alterations positioning it as a promising candidate for a novel predictive and prognostic biomarker in AKI patients(Younes-Ibrahim and Younes-Ibrahim, 2022).

FGF23 mainly affects the kidney, where it inhibits the transcription of 1α-hydroxylase, the essential enzyme for the activation of vitamin D hormone (1,25(OH)2D), and promotes the transcription of 24-hydroxylase, the primary enzyme for vitamin D breakdown, in proximal renal tubules. The circulating concentration of 1,25(OH)2D positively regulates FGF23 secretion in bone, establishing a feedback loop between the kidney and bone (Latic and Erben, 2021). Individuals with chronic kidney disease (CKD) exhibit significantly elevated levels of FGF23 and are likely the demographic experiencing the most significant detrimental effects associated with FGF23 (Rodelo-Haad *et al.*, 2019). FGF-23 concentrations increase rapidly in patients undergoing maintenance haemodialysis. A strong correlation exists between FGF-23 and levels of phosphorus and PTH, No correlation between FGF-23 and mortality in haemodialysis patients (Anandh *et al.*, 2017).

In chronic kidney disease (CKD), FGF23 levels are enhanced compared to those in healthy individuals. Increased FGF23 levels are negatively correlated with iron-deficient anaemia in CKD patients, demonstrating excellent sensitivity and specificity, thereby positioning FGF23 as a viable biomarker for predicting disease progression (Mousa *et al.*, 2022). Elevated FGF-23 levels appear to be independently correlated with mortality in patients initiating haemodialysis treatment. Future research may explore the potential of FGF-23 as a biomarker to inform strategies for managing phosphorus balance in individuals with chronic renal disease (Gutiérrez *et al.*, 2008).

In prospective investigations, elevated serum FGF-23 levels predicted accelerated disease development in CKD patients not undergoing dialysis and heightened mortality in individuals on maintenance haemodialysis. FGF-23 may

thus serve as a significant therapeutic target in the treatment of chronic kidney disease (CKD) (Seiler, Heine and Fliser, 2009).

Initiation of hemodialysis in individuals with chronic renal failure resulted in a gradual decrease in FGF-23 levels, concomitant with decreases in serum phosphorus levels. indicate that phosphorus significantly stimulates FGF-23 production and that the control of FGF-23 synthesis occurs rapidly (Kawabata *et al.*, 2020).

1.2.8. Complications associated with hemodialysis

Hemodialysis patients are at increased risk of infections, which are common adverse events among this patient population. (Nguyen, Arduino and Patel, 2019) Bacterial infections are common causes of mortality and morbidity among chronic kidney disease (CKD) patients under hemodialysis and infectious disease is the second most common cause of death in patients receiving hemodialysis (HD).(Villalon, Farzan and Freeman, 2018). Patients undergoing hemodialysis (HD) are at an increased risk of bacterial infections, and they are associated with high morbidity and mortality rates.(Mei Tao Danna Zheng and Zhang, 2022).

There are several risk factors for the development of bloodstream infection in patients with HD. These risk factors include the use of a central venous catheter (CVC), having diabetes mellitus, hypoalbuminemia, and anemia, and female sex. Also, colonization by methicillin-resistant Staphylococcus aureus (MRSA) is effective in this process (Samarbaf-Zadeh *et al.*, 2015).

Urinary tract infections (UTIs) which are common in dialysis patients, are associated with increased rate of complications, and may be difficult to diagnose due to often subclinical presentation.(Manhal, Mohammed and Ali, 2012)

Infection is the second cause of death in dialysis patients with terminal renal failure. The causes for infection during hemodialysis are varied and are mainly classified as the patient's inherent factors and iatrogenic factors. These two classes of factors can exist individually and interact with each other.(Wang, 2017). Sources of infections include contaminated water, equipment, environmental surfaces, and infected patients. Contaminated healthcare worker hands are among the most common modes of transmission of healthcare-associated infections.(Karkar, 2018). Inflammation is highly prevalent in chronic hemodialysis patients. Because hemodialysis involves the contact of blood with "foreign" surfaces, and the documented activation of several humoral and cellular pathways during the procedure, the hemodialysis procedure has been suggested as a potential source of inflammation in this patient population. Earlier studies did not provide clear-cut evidence of the potential contribution of the hemodialysis procedure to inflammation, as assessed by markers of inflammation such as cytokine levels and acute-phase protein production(Caglar *et al.*, 2002).

1.2.8.1. UTI in hemodialysis patients

Urinary tract infection (UTI) is the microbial invasion of any tissues within the urinary tract, which extends from the renal cortex to the urethral meatus. The urinary tract comprises the organs that collect, store, and release urine, including the kidneys, ureters, bladder, urethra, and associated structures. Urine produced in the kidneys is a sterile fluid that provides an optimal culture medium for the development of bacteria, leading to urinary tract infections (UTIs) caused by microorganisms including bacteria, viruses, fungi, and parasites that damage the upper or lower urinary tract (Haider, 2016). The urinary tract contains the urethra, bladder, ureters, and kidneys. Untreated urinary tract infections can result in

consequences including sepsis, renal damage or scarring, and renal infections (Onyebueke, Onyemelukwe and Oladeji, 2019).

Infectious diseases affect humans throughout all age groups and typically require prompt treatment. Asymptomatic urinary tract infections are more prevalent in haemodialysis patients with chronic renal failure due to their compromised defence mechanisms. A urinary tract infection (UTI) is clinically defined as the presence of bacteria multiplying in urine at a concentration exceeding 10^5 colony-forming units (CFU) or organisms per millilitre in a clean catch midstream urine sample (Chaudhary, 2016).

Escherichia coli is the predominant bacteria responsible for urinary tract infections in the majority of individuals. Recurrent urinary tract infections (RUTI) are primarily caused by reinfection by the same organism (Al-Badr and Al-Shaikh, 2013). Individuals are susceptible to urinary tract infections (UTIs), which may result in severe consequences. The challenges in obtaining urine samples from anuric or oliguric haemodialysis patients complicate the initial diagnosis of urinary tract infections (Bahramian *et al.*, 2021).

A urinary tract infection (UTI) can occur when bacteria infiltrate the bladder or kidney and proliferate in the urine. The urethra is shorter in females than in males, facilitating the ascent of bacteria to the bladder or kidneys, hence increasing the risk of infection (Tan *et al.*, 2022).

1.2.8.2. Expected isolated bacteria from urinary tract infection:

The most frequent causative agent of UTI is bacteria where aerobic Gram-negative bacilli have a predominant contribution. In addition, Gram-positive cocci also contribute to a considerable portion of UTIs.(Gebretensaie *et al.*, 2023). Bacterial species causing UTI are caused by Gram-negative bacteria, followed by

Gram-positive bacteria. Most commonly by Escherichia coli, *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterococcus faecalis* and *Staphylococcus saprophyticus*.Women are at greater risk of developing a UTI than men (Dhanashri Santosh Mane and Pramod Motiram Bhosale, 2023).

1.2.8.2.1: Gram-negative bacteria that caused UTI

Escherichia coli is still the most common pathogen in UTIs. A wide variety of other pathogens may be involved in UTI.(Scherberich, Fünfstück and Naber, 2021) The majority of UTIs are caused by uropathogenic *Escherischia coli* (UPEC), which can bind to urothelial cells, after which it is either expelled or invades into the cytoplasm. Exfoliation of the host urothelial can hasten recovery by increasing excretion of bacteria-filled cells via the urine, but can also aid deeper infiltration of UPEC into the urothelium, resulting in persistent infection. The exfoliation process has been shown to be dependent on caspases, but a deeper understanding of the mechanism in *E. coli* has remained elusive(Fenner, 2015).

Klebsiella pneumoniae is the most relevant human pathogen within the genus *Klebsiella*, causing many infections in hospitals(Cristea *et al.*, 2017). Urinary tract infection (UTI) associated with *Klebsiella pneumoniae* poses a serious threat for inpatients. *K. pneumoniae* is a significant causative agent of UTI, which can result in high rates of illness and death (Wei Zhang *et al.*, 2024).

Proteus mirabilis is a Gram-negative bacterium and this organism is most frequently a pathogen of the urinary tract.(N. and M., 2015) *Proteus* rods are opportunistic bacterial pathogens that under favorable conditions cause urinary tract infections (UTIs), commonly associated with complicated urinary tract infections7 . They generally affect the upper urinary tract (common site of infection), causing infections such as urolithiasis (stone formation in kidney or

bladder), cystitis, and acute pyelonephritis. Rare cases of bacteraemia, associated with UTIs, with *Proteus s*pp. (Mohammed, Kadhim and Hameed, 2016)

1.2.8.2.2. Gram-positive bacteria that caused UTI

Gram-positive bacteria are said to be responsible for ten percent of urinary tract (UTI) infections (Gajdács *et al.*, 2020). Gram-positive microorganisms are generally less frequent in urinary tract infections (UTIs). However, they should be considered as possible agents (Fernández-Espigares *et al*., 2023).

The predominant gram-positive bacteria are *Staphylococcus spp*., including *Staphylococcus aureus* and coagulase-negative *staphylococci* (CoNS) (Aniba *et al.*, 2024)

Enterococcus faecalis has been commonly considered as one of the major pathogens of urinary tract infections (UTI) in the human host worldwide (Ma *et al.*, 2021). *Enterococcus spp*. is rank third among the most common pathogens isolated from intensive care unit patients with UTIs , and are a common cause of chronic or recurrent UTIs (Nathan *et al.*, 2001).

Chapter Two

Subjects ,Materials and Methods

2. Subjects, Materials and Methods

2.1. Study Design and Setting

Subject This case–control study was done at Habib Ibn Mudhaher Dialysis Center in Imam AL Hussein Teaching Hospital in Kerbala. All patients were registered in the hospital from October 2024 to January 2025.

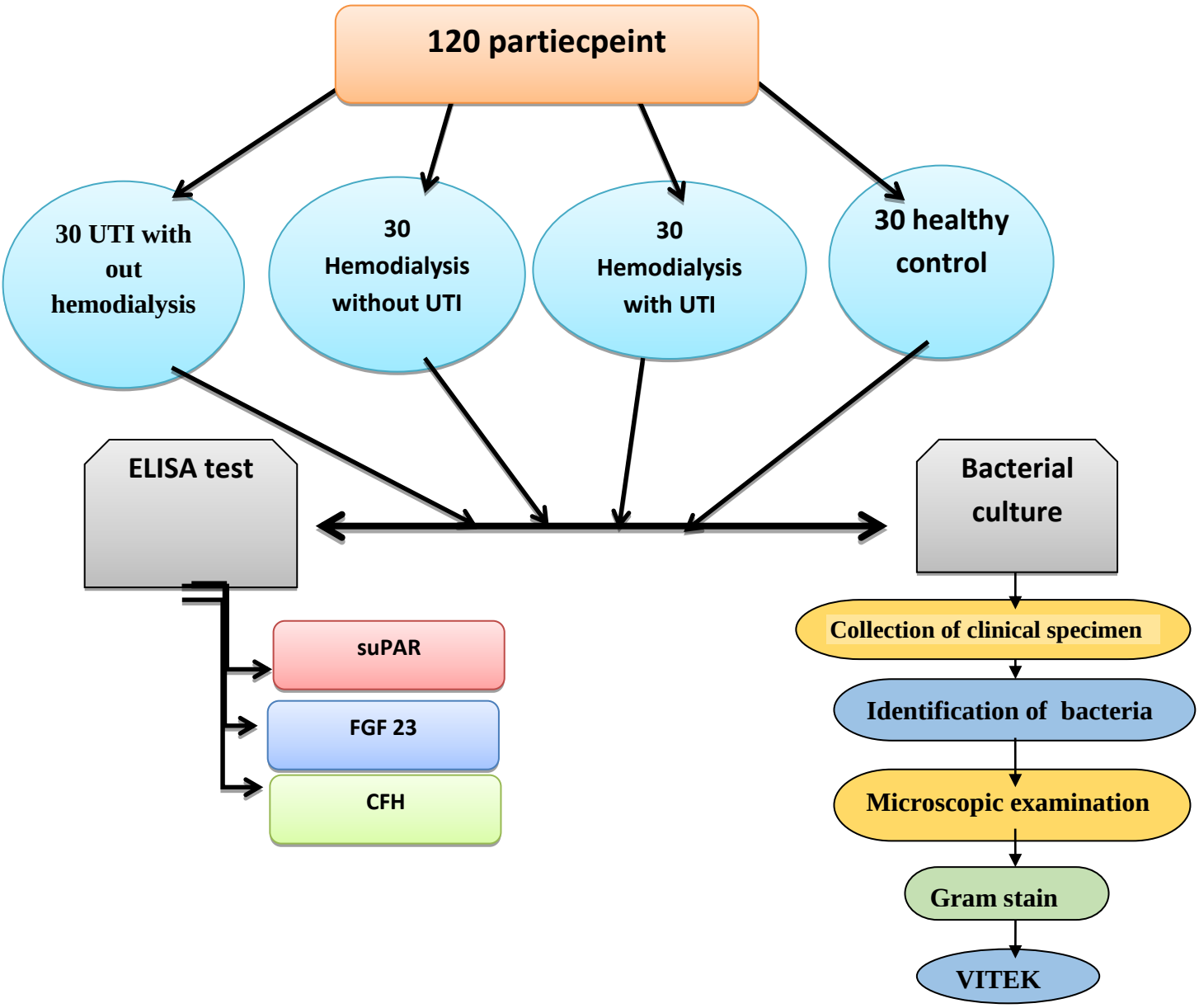

Figure 2.1 : Basic Design Of The Study

2.2 Subject Group

one hundred twenty (120) participants were enrolled in this study, including three groups involved in this case-control study according to clinical diagnosis by a clinician, divided into :

Group I: 30 patients on hemodialysis with UTI. Group II: 30 patients on hemodialysis without UTI. Group III:30 patient not on hemodialysis with UTI.

Compared with the 30-healthy control group. Detailed case information of all the groups , including age, sex, and other variables collected for each patient by a questionnaire as appendix1.

2.3. Inclusion Criteria

Patients with renal failure were diagnosed on the basis of clinical symptoms and other investigations under hemodialysis for at least three months, male and female with different age groups.

2.4. Exclusion Criteria

Patients who have cancer, drugs with immunosuppression, autoimmune diseases, allergies, asthma, and patients with foley catheterization, were excluded, for the control group, the same as mentioned above will also be excluded.

2.5. Designation of Questionnaire for Patients

A total of 60 hemodialysis patients were registered at the Habib Ibn Mudhaher Dialysis Center in Imam Hussein medical city in Kerbala. In addition, 30 patients with urinary tract infections (not on hemodialysis) were included from the nephrology department. Patients with renal failure were interviewed using a specially designed questionnaire that collected demographic data, including age, sex , medical history, living situation, telephone number, family history, and antibiotic

usage. Urine samples were collected whenever possible; however, this was often difficult because many dialysis patients experience significantly reduced urine output.

2.6. Ethical Approval

The study protocol was sent to the ethical committee in medicine collage of Kerbala university and the relevant ethical committees in Kerbala Health Directorate (3772). Verbal approval was obtained from the patients' relatives before collecting the samples. All necessary health and safety measures were implemented during sample collection.

2.7. Materials

2.7.1. Equipment and Instruments Utilized in the Study

In the present study, the following equipment and instruments were used in table (2.1).

Table (2.1): Devices and Instruments.

Equipment & Instruments	Manufacturing Company	Origin
70% alcohol swabs for skin disinfection.	trest	chins
A drying rack.	. ALS	. China
A micro-pipette.	SLAMED	Germany
A sterile glass (gel tube)&(plain tube)	ALS	China
A tourniquet.	Himedia	India
Autoclave	Hirayama HVE-50	Japan
Biological safety cabinet	EuroCloneSafemate	Italy
Burner	Amal	Turkey
Centrifuge	Kokusan	Japan
Deep freezer	Hettich	
ELIZA Devices (washer & reader)	Human	Germany
ELIZA printer	Epson	Japan
flasks (different size)	Jlassco	India

gauze or cotton-wool ball to be applied over puncture site.	BDH	England
Hemo-Cue Hb 301	HemoCue AB	Sweden
Incubator	Memmert	Germany
Light microscope	Olympus	Japan
Loop	Himedia	India
maker	trest	china
Multichannel micropipette set	SLAMED	Germany
Para-film	Bemis	USA
pipette tips.	ALS	China
Refrigerator	Panasonic	Korea
Sensitive balance	Sartorius	Germany
Slides	Himedia	India
Swab media		
Syringe 5 ml	Arrow	Egypt
VITEK 2 compact system	Bio merieux	France
vortex	Clay Adams	Germany
Water bath	Polyscience	USA
water distillatory	GFL	Germany
well-fitting gloves.	ALS	China

2.7.2. Chemical Materials:

The chemical materials are listed in table 2.2.

Table (2.2): Chemicals and biological materials that are used in the study.

Chemical materials	Company	Origin
Normal saline (0.9	choueifat	Lebanon
Gram's stain kit	Biolife	Italy
Glycerol		
Oil immersion	BDH	England

2.7.3. Culture media :

The culture media used in the present study are in table (2-3).

Table (2.3): Culture media used in the current study.

Culture media	Company	origin
Blood agar base (BAB)	Himedia	India
Brain heart infusion broth (BHIB)	Oxoid	England
MacConkey agar		
Mannitol salt agar		
Chocolate Agar		

2.7.4.Commercial kits:

The commercial kits used in the present study are listed in table (2.4).

Table (2.4.): The commercial kits which are used in the study

Kits	**Company**
Fibroblast growth factor 23 (FGF 23) ELISA Kit	BT LAB / China
Human soluble urokinase plasomongen activator recopter (suPAR) ELISA Kit	
Complement factor H (CFH) ELISA Kit	
ID (VITEK2) cards cassette	BioMerieux /France
Hb kit	Hemocue301/Sweden
Serum phosphorus kit	Cobs/Roche, Germany
Serum calcium kit	
Serum sodium kit	
B.urea kit	

Serum creatinine kit	
Serum ferritin kit	Abbott/Germany
PTH kit	
UIBC kit	
Iron kit	
Serum phosphorus kit	Cobs/Roche, Germany
Serum calcium kit	

Table (2.5.): Reagents and Quantity (suPAR) ELISA Kits.

Components	Quantity (96T)	Components
Biotinylated Human Supar Antibody	1ml x1	1ml x1
Plate Sealer	2 pics	2 pics
Pre-coated ELISA Plate	12 * 8 well strips	12 * 4 well strips
Standard Diluent	3ml x1	3ml x1
Standard Solution (240ng/L)	0.5ml x1	0.5ml x1
Stop Solution	6ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Substrate Solution A	6ml x1	3ml x1
Substrate Solution B	6ml x1	3ml x1
User Instruction	1	1
Wash Buffer Concentrate	20ml x1	20ml x1
Zipper bag	1 pic	

Table (2.6): Reagents of Human FGF 23 ELISA Kits

Components	Quantity (96T)	Quantity(48T)
Biotinylated Human FGF 23 Antibody	1ml x1	1ml x1
Plate Sealer	2 pics	2 pics
Pre-coated ELISA Plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard Diluent	3ml x1	3ml x1
Standard Solution (240ng/L)	0.5ml x1	0.5ml x1
Stop Solution	6ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Substrate Solution A	6ml x1	3ml x1
Substrate Solution B	6ml x1	3ml x1
User Instruction	1	1
Wash Buffer Concentrate	20ml x1	20ml x1
Zipper bag	1 pic	

Table (2.7): Reagents of Human CFH ELISA Kits

Components	Quantity(96T)	Quantity(48T)
Biotinylated Human CFH Antibody	1ml x1	1ml x1
Plate Sealer	2 pics	2 pics
Pre-coated ELISA Plate	12 * 8 well strips x1	12 * 4 well strips x1
Standard Diluent	3ml x1	3ml x1

Standard Solution (240ng/L)	0.5ml x1	0.5ml x1
Stop Solution	6ml x1	3ml x1
Streptavidin-HRP	6ml x1	3ml x1
Substrate Solution A	6ml x1	3ml x1
Substrate Solution B	6ml x1	3ml x1
User Instruction	1	1
Wash Buffer Concentrate	(25x) 20ml x1	20ml x1
Zipper bag	1 pic	

2.8. Method

2.8.1 Biological Sampling

2.8.1.1. Urine sample

Clean-catch midstream urine specimens were collected from study patients in a sterile container and sent to the laboratory immediately. A typical medical urinalysis employing urine dip stick, microscopic examination and bacteriological culture were included according to standard methods. Bacteriuria is defined as the detection of ≥ 105 cfu/mL of a single microorganism in the culture of urine specimens . Pyuria is defined as the presence of ≥10 neutrophils per high-power field of voided midstream urine as used by most studies that evaluated the diagnostic relevance of pyuria in dialysis patients . Bacterial culture growth was counted according to the number observed per plate, fewer than 10 colonies per plate represented < 103 cfu/ml, 10 to 100 colonies per plate represented 103 to 104 cfu/ml, 100 to 1000 colonies per plate represented 104 to 105 cfu/ml, and > 1000 colonies per plate presented > 105 cfu/ml. Microorganisms isolated from all

specimens were identified at species level according to standard microbiology methods, that's including direct microscopic examination of gram stained preparations and biochemical profiling tests.(NCBI Bookshelf, 2023)

2.8.1.2. Blood Sample.

Venepuncture is the preferred method of blood sampling. Use a syringe with a barrel volume of 5 ml, depending on collection needs; the vacuum produced by drawing using a larger syringe will often collapse the vein. Collect all the equipment needed for the procedure and place it within safe and easy reach on a tray or trolley, ensuring that all the items are clearly visible. (World Health Organization 2010)

2.8.2. Laboratory Methods

2.8.2.1. Serum preparation

After drawing a sufficient amount of whole blood into a serum gel tube, gently invert the tube several times to activate clotting. Allow the blood to clot at ambient temperature (20–30 minutes). Centrifuge the sample for 5 minutes to separate the serum from the clot, then transfer the serum to a screw-capped plain tube. This process must be completed within 1 hour of specimen collection for optimal results.

2.8.2.2. culturing Media (Streak method)

For the isolation of bacteria in pure culture from clinical specimens. One swab full of the specimen is transferred onto the surface of a well-dried plate. Spread over a small area at the periphery. The inoculum is then distributed thinly over the plate by streaking it with a loop in a series of parallel lines in different

segments of the plate. On incubation, separated colonies are obtained over the last series of streaks**.** (Forbes, Sahm & Weissfeld 2014)

Pure Culture: Culture consists of a medium containing the growth of a single species of M.O., which is involved in most microbiological studies and industries. (Forbes, Sahm & Weissfeld 2014)

Mixed Culture: Culture consists of a medium containing the growth of two or more species of M.O. There are several ways to ensure the purity of a culture.

If the mixture of various nutrients that is suitable for the growth of microorganisms. This media should provide bacteria with most of important nutrition requirements for growing such as water, source of carbon , energy , nitrogen , carbohydrates , amino acid , inorganic salts , particular growth factors in some time like vitamin (K) and others . These nutrients are supplied by aqueous extracts of meat and peptone . (Forbes, Sahm & Weissfeld 2014)

2.8.2.3. Media used culturing bacteria

A: Blood Agar

A nonselective medium for the growth of harmful bacteria, such as *streptococci*, is blood agar. It also acts as a medium for differentiation between hemolytic and non-hemolytic microorganisms. Hemolysis is often permitted in the medium. Cytolytic toxins are typically produced by hemolytic bacteria. In blood agar, these toxins have the power to lyse red blood cells. As a result, the medium receives the red component of the cells. The medium becomes stained or dark due to hemolysis. Actually, there are three different kinds of hemolysis: gamma hemolysis, which leaves the medium uncolored, beta hemolysis, which turns the medium clear, and alpha hemolysis, which turns the medium dark or stained as in figure 2.1.

Figure 2.2: Hemolysis of blood agar . L G Reimer(2001)

B: Chocolate Agar

Another nonselective, enriched medium that's crucial for isolating harmful bacteria is chocolate agar. The primary characteristic of chocolate agar is the presence of heated red blood cells that have been lysed. Chocolate agar is essential for the growth of bacteria that are picky eaters, like *Neisseria meningitidis* and *Haemophilus influenzae*. These bacteria typically need two internal growth factors found in red blood cells to proliferate. Factor V and Factor X are among them. Nicotinamide adenine dinucleotide is factor V; hemin is factor X. Therefore, the primary ingredient needed for these bacteria to develop is red blood cell lysate.

Definition: Blood agar refers to a general-purpose, enriched media to grow fastidious organisms and differentiate bacteria based on their hemolytic properties, while chocolate agar refers to a nonselective, enriched growth medium used for the isolation of pathogenic bacteria.

Red Blood Cells : Blood agar contains red blood cells of whole sheep blood, while chocolate agar contains red blood cells lysed by heating. **Color** :Blood agar is red in color, while chocolate agar is brown to brownish red in color.

Cooling Temperature :Sterile sheep blood is added at 50°C to nutrient agar in the preparation of blood agar, while sterile sheep blood is added at 75-80°C to nutrient agar in the preparation of chocolate agar.

Factor V and X :Factor V and X occur inside the red blood cells in blood agar, while Factor V and X occur in the medium in chocolate agar.

Type of Organisms :Blood agar is important for the growth of *S. pyogens* and other *streptococcus* species., while chocolate agar is important of *Neisseria* and *Haemophilus* species.

C: MacConkey Agar

Differential and selective media; *Enterobacteriaceae* identification contains peptone, lactose, and bile salts, which inhibit most Gram+ bacteria with the exception of *Enterococcus* and certain *Staphylococcus* species.

Indicators and Reagents: contains neutral red dye, which gives microorganisms that are fermenting lactose (and lowering pH) a pink stain, as well as crystal violet and bile salts, which suppress Gram+ bacteria.

Figure (2.3): MacConkey Agar (Sagar Aryal , 2015) .

Mechanism/reactions: *Enterobacter, Klebsiella,* and *Escherichia coli* are examples of Lac+ bacteria that use the lactose in the medium to create acid. This lowers the pH of the agar below 6.8 and causes red or pink colonies to develop. Since they are unable to use lactose, bacteria that do not ferment lactose, such as *Shigella*, *Proteus* species, and *Salmonella*, will instead use peptone. This results in the creation of ammonia, which elevates the agar's pH and causes white or colorless colonies to grow.

Interpretation:

(+) = Lactose fermentation, red/pink colonies.

(-) = non-lactose fermenters, white/colorless growth.

D: Mannitol Salt Agar

Differential and selective; pathogen detection The *staphylococci* include Mannitol, 7.5% Sodium Chloride, and Phenol Red in Mannitol Salt Agar (MSA) .Indicators and Reagents :Red phenol

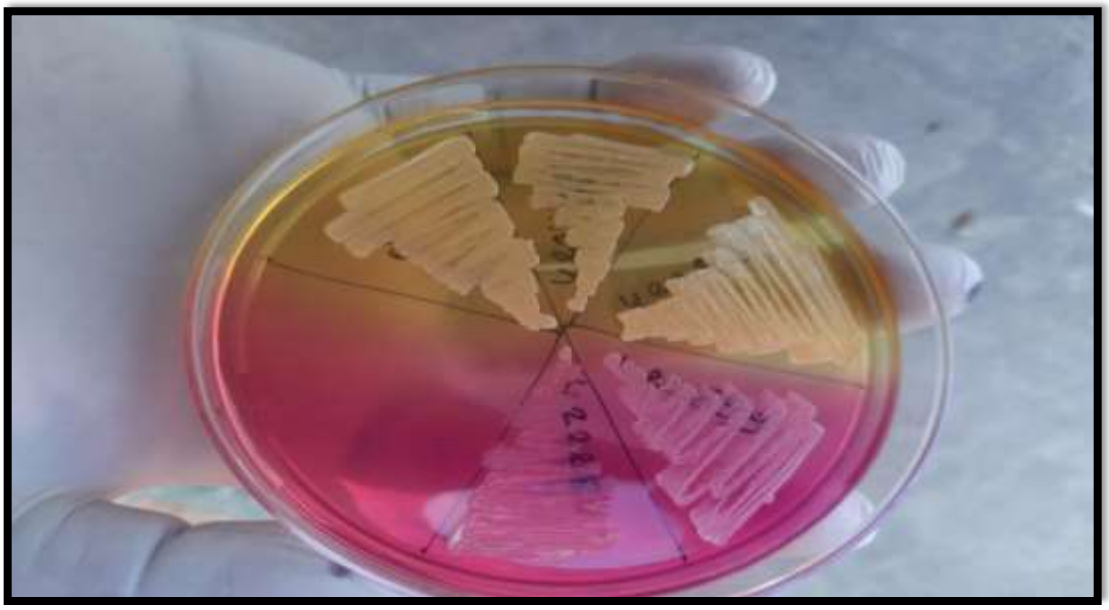

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Figure (2.4) : Mannitol Salt Agar (MSA) (mubashir iqbal, onmay 21, 2023)

Mechanism/reactions : Because the concentration of salt inhibits the majority of other organisms, the medium is *staphylococci*-specific. When *Staphylococcus aureus* ferments mannitol, the medium's pH changes to an acidic state, which may be seen by the color of phenol red turning yellow.

Interpretation:

(+) = Growth and yellow halo surrounding it (also record growth/no color)

(-) = No growth, no color change

E. Brain heart infusion .

BHI Medium is a highly nutritive medium that is used to cultivate a wide range of microorganisms. Both fastidious and non-fastidious microorganisms can

be grown in BHI Broth, an enhanced non-selective broth medium. Aerobic bacteria from a range of clinical and non-clinical specimens will also grow well in this medium. BHI is utilized to maintain microbiota for three months in deep freezing, allowing for the culture of bacterial samples on nutrient or blood agar for eventual Viteks system diagnosis. Component of BHI The mixture of brain and heart infusions provides organic nitrogen, carbon, and vitamins. Dextrose is the carbohydrate source. A low concentration of dextrose is used to stimulate early growth. Sodium chloride maintains the osmotic environment. Disodium phosphate is the buffering agent in this medium and also helps neutralize the acids produced from the utilization of dextrose, thus maintaining viability (Catrambone *et al.,* 2021).

2.9. Sterilization Methods

A: Sterilization of the culture media used in the present study by autoclave at 121Co for 15 minutes. (Madigan et al. 2019)

B: Sterilization of the glass wares are done by dry heat in an electric oven at 180 Co for 2 h. (Madigan et al. 2019)

2.10. Bacterial Profile Identification

2.10.1. Morphological Tests

Colonial characteristics were tested such as the shape of the colonies, size, color, borders, and texture of colonies.

2.10.2.Microscopic Characteristics

Following Gram stain, bacteria were seen under a light microscope. A tiny portion of a bacterium colony was applied on a clean slide using a drop of regular saline, exposed to flames to fix it, then covered in crystal violet, Iodine, alcohol,

and safranine to counterstain before being studied under an oil immersion lamp (Tille, 2017).

2.10.3. Identification by using automated methods [VITEK2] system:

The fastest and most precise techniques for identifying bacteria are automated ones. In order to provide a biochemical profile that is utilized for organism diagnosis, the VITEK2 system is made up of plastic reagent cards with microliter quantities of various biochemical test media in 30 wells (Maina and Kagotho, 2014). A photometer is used to evaluate the color changes in the card caused by the microbe's metabolic activity. An inoculum obtained from cultured samples is automatically placed into the card. A computerized data base was used for the analysis, storing, and printing of the data. Various card kinds, such as those for Gram-positive and Gram-negative identification (GN and GP), are available (Maina and Kagotho, 2014). The phases of the VITEK 2 compact system are indicated in appendix 4.

2.11. Maintenance of bacterial isolates

The bacterial isolates' maintenance was done as follows :

2.11.1. Short-Term Storage

Pure bacterial isolates were stored for a few months in screw-capped universal tubes containing brain heart infusion (BHI) agar slants. These slants were incubated at 37°C for 24 hours. After incubation, the slants were tightly sealed with Parafilm and stored at 4°C for up to three months, as outlined by (Benson, 2007)

2.11.2. Long-Term Storage

For long-term storage, a loop of overnight pure bacterial culture was inoculated into brain heart infusion (BHI) broth and incubated at 37°C for 18 hours. After incubation, glycerol was added to the broth to achieve a final concentration of 20%. The mixture was then stored at -20°C for a period of 2 to 8 months (Green, 2015).

2.12. Hematological and biochemical assays

2.12.1. Estimation of Hemoglobin (Hb)

Together with microcuvettes, the system has an analyser. The micro-cuvette acts as both a pipette and a measuring cuvette. A sample of about 10 microliters of blood is taken into the cavity by capillary action. The measurement is performed in the analyzer, which assesses the absorption of whole blood at an isobestic point of Hb / HbO2. To account for turbidity, the analyser scales at two wavelengths (506 and 880 nm). The Hemo-Cue Hb 301 device is measured against the form hemi-globin-cyanide (HiCN), the universal reference form for assessing the concentration of hemoglobin in the blood. The system is calibrated by the factory and does not need any further calibration (Whitehead *et al.*, 2017).

2.12.2. Estimation of Serum Parathyroid Hormone (PTH)

The architect PTH biomarker is a two-step immune assay using Chemiluminescent Microparticle Immune-Assay (CMIA) technology for quantitative determination of intact PTH in human serum and plasma. Sample, diluent assay, and anti-PTH-coated paramagnetic micro-particles are combined in the first step. PTH in the sample binds to the micro-particles which are coated with anti-PTH. In the second step, anti-PTH acridinium-labelled conjugate is added after

washing to create a reaction mixture. After another wash cycle, pretrigger and trigger solutions are added to the mixture of reactions. The resulting chemical luminescent reaction is evaluated as units of relative light (RLUs). The PTH architect is suitable for use with both STAT and Routine guidelines. The STAT protocol has a shorter time of incubation compared to the Routine Protocol. Because of different incubation times, the routine and STAT protocols need separate calibrations but need only one reagent kit. There is a clear relationship between the sum of PTH in the sample and the RLUs that architect I System optics detect

Reference range for healthy adults (15-68.3) pg/mL (Kuczera *et al.*, 2015).

2.12.3. Estimation of B.Urea

Urea is measured enzymatically using the urease-glutamate dehydrogenase (GLDH) method. Urease hydrolyzes urea into ammonia and carbon dioxide. The ammonia reacts with α-ketoglutarate and NADH in the presence of GLDH, resulting in a decrease in NADH absorbance. This decrease is measured photometrically at 340 nm and is directly proportional to the urea concentration. Abbott analyzers perform this method automatically with high precision.(Abbott Ireland Diagnostics Division 2022)

2.12.4. Estimation of S.creatinine

Serum creatinine is measured using an enzymatic method on Abbott analyzers. Creatinine is converted to creatine by creatininase, then to sarcosine by creatinase. Sarcosine is oxidized by sarcosine oxidase, producing hydrogen peroxide, which reacts with a dye in the presence of peroxidase to form a colored compound. The absorbance is measured photometrically, and the intensity is

directly proportional to the creatinine concentration..(Abbott Ireland Diagnostics Division 2022)

2.12.5. Estimation of Serum Iron

At a ph of 4.8, iron is set to release from the transferrin , and then reduced quantitatively to a ferrous state. The iron form with ferene S (3-2pyridyl-5,6-bis2(5-furylssulfonic acid)-1,2,4-triazine), a stable colored complex of which the iron content in the sample is proportional to the intensity of color. Particular reaction state and unique masking agent remove the copper interference almost entirely.

Reference range for males is (65-175) microgram/dL, females (50-170) microgram/dL (Hedayati *et al.*, 2018) .

2.12.6. Estimation of Serum Ferritin

The ferritin assay by the architect is a 2-step immuno-assay to assess the existence of ferritin in human serum using CMIA technology with flexible assay guidelines called chemiflex combining the sample and anti-ferritin-coated paramagnetic micro-particles. The ferritin present in the sample binds to the anti-ferritin coated micro-particles. Added to create a reaction mixture after washing anti-ferritin acridinium -labeled conjugate. The reaction mixture is added to the pretrigger and trigger solution after another wash cycle. The resulting chemical luminescent reaction is measured as units of relative light (RLUs). The amount of ferritin in the sample has a direct relationship with the RLUs detected by the architect i system optics. Reference range for males is (21.81-274.66) ng/ml, females is (4.63-204) ng/mL (Bleicher *et al.*, 2018).

2.12.7. Estimation of Serum UIBC

Sample is an addition to an alkaline buffer containing a specific concentration to saturate the binding site available on the transferrin. After

transferrin saturation is reduced to a ferrous state, the iron remains free and then complexed by ferene S (3-2pyridyl-5,6-bis-2(5-furylssulfonic acid)1,2,4-triazine) to form a stable complex, intensity is measured at 604 nm. The intensity of the color is directly proportional to the unbound excessive iron concentration and indirectly proportional to the unbound iron quantity from the total add amount. Reference range for male is (69-240), female (70-310) microgram/dL(Obeagu *et al.*, 2016).

2.12.8. Estimation of Serum Phosphorus (PO4)

Endpoint technique with blanking of a sample. Inorganic phosphate forms an ammonium phosphomolybdate complex with the ammonium-molybdate formula (NH4)3(PO4(MoO3)12) in the presence of sulfuric acid.

$$\text{Phosphate+ammonium-molybdate} \xrightarrow{H2SO4} \text{ammonium phosphor-molybdate}$$

The concentration of formed phosphorus molybdate is directly proportional to the concentration of inorganic phosphate and is photo-metrically calculated. Reference range in adults is (2.5-4.5) mg/dl (Kiran *et al.*, 2014) .

2.12.9. Estimation of Serum Calcium

In alkaline condition, the calcium ion reacts with 5nitro-5methyl-BAPTA(NM-BAPTA) to form a complex. In the second step this complex was reacted to with EDTA.

$$Ca+2 + \text{NM- BAPTA} \xrightarrow{\text{alkaline ph}} \text{calcium-NM-BAPTA complex}$$

$$\text{Calcium -NM-BAPTA complex} + \text{EDTA} \rightarrow \text{NM-BAPTA} + \text{calcium EDT Complex}$$

The absorbance change is directly proportional to the concentration of calcium, and is measured photo-metrtically. Reference range for adults is (8.6-10) mg/dl (Bazydlo, Needham and Harris, 2014) .

2.12.10. Estimation of Serum Sodium

Sodium ions (Na^+) are measured photometrically using a chromogenic reagent that forms a colored complex with Na^+ in an alkaline medium. The absorbance is measured at a specific wavelength (typically 500–600 nm) and is directly proportional to the sodium concentration. A calibration curve using sodium standards is used for quantification. The normal reference range for serum sodium is 135–145 mmol/L(Bazydlo, Needham and Harris, 2014) .

2.13. Determination of Immunological Markers (CFH, suPAR, FGF23)

The levels of Complement Factor H (CFH), soluble urokinase plasminogen activator receptor (suPAR), and Fibroblast Growth Factor 23 (FGF23) were measured using commercially available Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits (CFH: Cat. No. E0324Hu, suPAR: Cat. No. E3759Hu, FGF23: Cat. No. E0059Hu; BT Laboratory, China).

Principle

The ELISA kits are based on the sandwich technique. The microplate wells are pre-coated with a specific antibody for each marker. The target antigen present in the sample binds to the immobilized antibody. A biotinylated detection antibody specific for the same antigen is then added, followed by streptavidin conjugated to horseradish peroxidase (HRP). After washing to remove unbound reagents, a substrate solution (TMB) is added, producing a color change proportional to the concentration of the marker. The reaction is stopped by an acidic solution, and the absorbance is read at 450 nm using a microplate reader.(BT Laboratory, China).

Reagent Preparation

• All reagents were brought to room temperature before use.

• Standards: Each kit contained a lyophilized standard. The standard was reconstituted and serially diluted 1:2 with standard diluent to obtain a concentration range appropriate for each marker (e.g., CFH: 800–50 pg/ml; suPAR: 960–60 ng/L; FGF23: 800–50 pg/ml). The standard diluent served as the zero standard (0).

• Wash buffer: Prepared by diluting the 25× concentrated solution with deionized water to obtain 1× working solution.

Assay Procedure

1. All reagents, standards, and samples were prepared according to the manufacturer's instructions and equilibrated to room temperature.

2. The required number of microplate strips was placed in the plate frame; unused strips were stored at 2–8°C.

3. Standards and samples were added to the wells (50 µl/well). For sample wells, detection antibody was also added.

4. Streptavidin-HRP (50 µl) was added to all wells except blank controls. The plate was covered with a sealer and incubated at 37°C for 60 minutes.

5. The plate was washed five times with 1× wash buffer, ensuring each well was soaked for 30–60 seconds.

6. Substrate solutions A and B were added sequentially (50 µl each) to all wells and incubated at 37°C for 10 minutes in the dark.

7. Stop solution (50 µl) was added, changing the color from blue to yellow.

8. The optical density (OD) was measured at 450 nm within 10 minutes using a microplate reader.

Calculation of Results

A standard curve was constructed by plotting the mean OD values of the standards (y-axis) against their concentrations (x-axis). The concentrations of CFH, suPAR, and FGF23 in the samples were calculated by regression analysis using curve-fitting software.

2.14. Statistical analysis

The statistical analysis was performed with IBM SPSS Statistics version 25. The results from the analysis were subsequently summarized adopting descriptive statistics. Likewise, the Mean and Standard Deviation have been determined. A probability criterion of $p < 0.05$ was utilized to evaluate the statistical significance of the experimental findings. Moreover, the Shapiro-Wilk test has been employed to determine the normality of the data, and the Levene test has been conducted to examine the homogeneity of variance. Additionally, the Mann-Whitney Test and Independent T-Test were utilized to determine statistical differences between two distinct groups. The analysis of variance (ANOVA) was employed to do multiple comparisons among the groups. Scheffé's post-hoc test was utilized at a significance threshold of $p < 0.05$ to do multiple comparisons among groups. Asterisks represent data which has a P value below 0.05.

Chapter Three
Results

3. Results :

3.1. Demographic Characteristics of Study Groups

The study groups exhibited significant differences in age and sex across the three patient categories (UTIs, renal failure [RF] without UTI, and RF with UTI) compared with the control group.

As shown in table 3-1, the age distribution among patient groups was not statistically significant (p = 0.2646). The UTI group had the highest proportion of individuals under 40 years of age 13(43.33%), followed by those aged 60 and above11 (36.67%), and those aged 40–59 6(20.00%). In the RF without UTI group, both the patient under 40 and the 40–59 age categories accounted for 12(40.00%) each, while those aged 60 and above comprised 6(20.00%). The RF with UTI group had the highest proportion of individuals aged 40–59 12(40.00%), followed by those aged 60 and above 10(33.33%), and under 40, 8(26.67%). Overall, the majority of participants were under 40 years of age, comprising 36.67% of the total, as presented in table 3-1.

Sex distribution among patient groups showed a statistically significant difference (p = 0.0004). In the UTI group, females were more susceptible to infection, representing 21(70.00%)of the group, compared to 9(30.00%) males. In the RF without UTI group, males predominated 23 (76.67%) over females 7(23.33%). In contrast, in the RF with UTI group, females constituted the majority 19(63.33%), while males accounted for 11(36.67%). Across all patient groups, the overall cohort had a nearly equal sex distribution, with 43(47.78%) males and 47(52.22%) females, as shown in table 3-1.

Table 3-1: Distribution of Patient Sex and Age Groups, Detailing the Levels and Percentage Representation

Parametes	Level	UTI	RF without UTI	RF with UTI	Total	P value
Age Group	< 40	13(43.33%)	12(40.00%)	8 (26.67%)	33(36.67%)	0.2646
	40 - 59	6(20.00%)	12(40.00%)	12 (40.00%)	30(33.33%)	
	>= 60	11(36.67%)	6 (20.00%)	10(33.33%)	27(30.00%)	
Sex	**Male**	9(30.00%)	23(76.67%)	11(36.67%)	43(47.78%)	0.0004
	Female	21(70.00%)	7 (23.33%)	19(63.33%)	47(52.22%)	
The chi-square test.*. Association is significant at the 0.05 level.						

3.2: Distribution of Pathogens among patient groups :

Culture investigations, based on morphological, biochemical, and VITEK 2 Compact system results, showed a statistically significant difference in the overall distribution of pathogens (p = 0.001). *Escherichia coli* was the most commonly isolated organism, accounting for 30 isolates (50.00%), followed by *Klebsiella pneumoniae* with 10 isolates (16.67%), and *Staphylococcus aureus* with 7 isolates (11.67%). Other identified pathogens included *Enterococcus faecalis* (3 isolates; 5.00%), *Pseudomonas aeruginosa* (3 isolates; 5.00%), and *Proteus mirabilis* (3 isolates; 5.00%). Additionally, single isolates (1 each; 1.67%) were identified for *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus saprophyticus*, and *Acinetobacter baumannii*, as shown in table 3-2.

Table 3.2: Distribution of Pathogens, Detailing the Levels and Percentage Representation

Types of pathogens	UTI	RF with UTI	Total
Escherichia coli	15(50%)	15(50%)	30(50.00%)
Klebsiella pneumoniae	6(20.00%)	4(13.33%)	10(16.67%)
Staphylococcus aureus	2(6.67%)	5(16.67%)	7(11.67%)
Enterococcus faecalis	2(6.67%)	1(3.33%)	3(5.00%)
Pseudomonas aeruginosa	2(6.67%)	1(3.33%)	3(5.00%)
proteus mirabilis	1(3.33%)	2(6.67%)	3(5.00%)
Staphylococcus haemolyticus	1(3.33%)	0.00%	1(1.67%)
Staphylococcus hominins	1(3.33%)	0.00%	1(1.67%)
Staphylococcus saprophyticus	0.00%	1(3.33%)	1(1.67%)
Acinetobacter baumannii	0.00%	1(3.33%)	1(1.67%)
P value	0.0002	0.0003	0.001
The chi-square test .*. Association is significant at the 0.05 level.			

3.3. Distribution of CFH between patient and control

The CFH parameter showed a statistically significant difference across the study groups (p = 0.0006). The mean ± standard deviation of CFH levels was highest in the renal failure without UTI group (291.40 ± 199.58), followed by the renal failure with UTI group (222.48 ± 189.98). Lower levels were observed in the UTI group (59.19 ± 22.72), still higher than the control group (42.49 ± 14.97). Overall, CFH levels were elevated in patients with renal failure—regardless of UTI status—compared to both the UTI and control groups. as shown in table 3-4

Table 3-3 : The Comparisons between study Groups According to the CFH Parameter

Groups	Mean	Std. Deviation	P. value
Control	42.490	14.971	0.0006
UTI	59.185	22.716	
RF without UTI	291.399	199.580	
RF with UTI	222.480	189.977	
ANOVA and Kruskal-Wallis.*. The mean difference is significant at the 0.05 level. **. The mean difference is significant at the 0.01 level.			

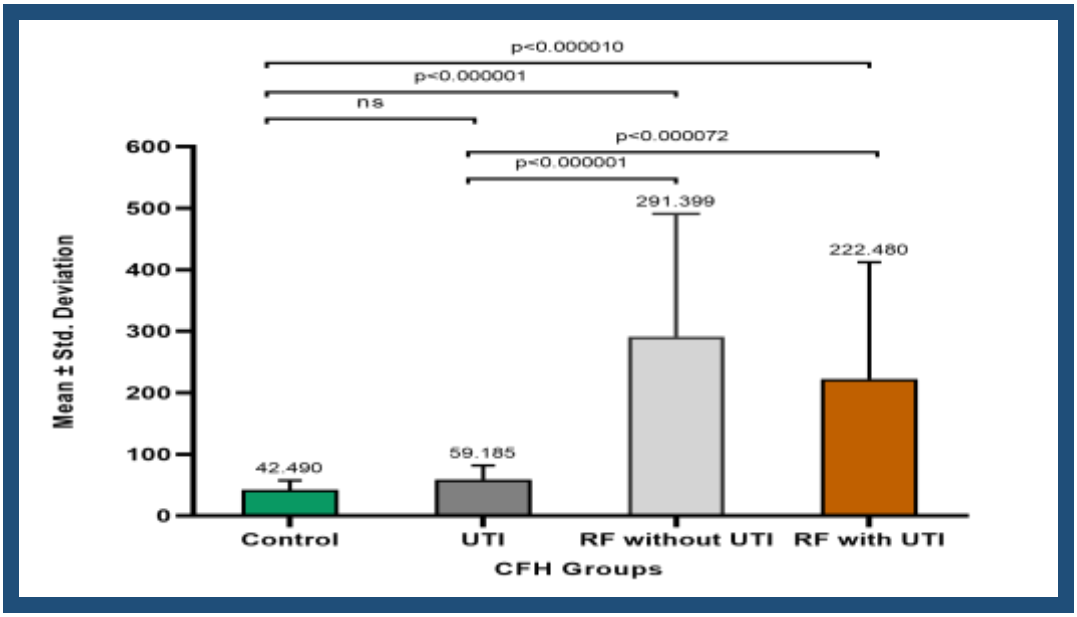

Figure 3-1: The Comparisons between study Groups According to the CFH Parameter.

3.4. Distribution of suPAR between patients and controls

The suPAR parameter demonstrates a statistically significant difference (P-value of 0.0002) when comparing various study groups. The mean and standard deviation of suPAR levels are highest in the renal failure group without urinary tract infections (UTI), with values of 441.802 ± 147.014. This is followed by the renal failure group with UTI, which has mean suPAR levels of 275.167 ± 258.077. The

UTI group has lower mean levels of 116.465 ± 58.729, although these are still elevated compared to the control group, which has mean levels of 75.529 ± 34.792. Overall, suPAR levels are elevated in individuals with renal failure, regardless of the presence of UTI, compared to both the control and UTI groups

Table 3-4 : The Comparisons between study Groups According to the suPAR Parameter

Groups	Mean	Std. Deviation	P. value
Control	75.529	34.792	0.0002
UTI	116.465	58.729	
RF without UTI	441.802	147.014	
RF with UTI	275.167	78.077	
ANOVA and Kruskal-Wallis tests.*. The mean difference is significant at the 0.05 level.. The mean difference is significant at the 0.01 level.**			

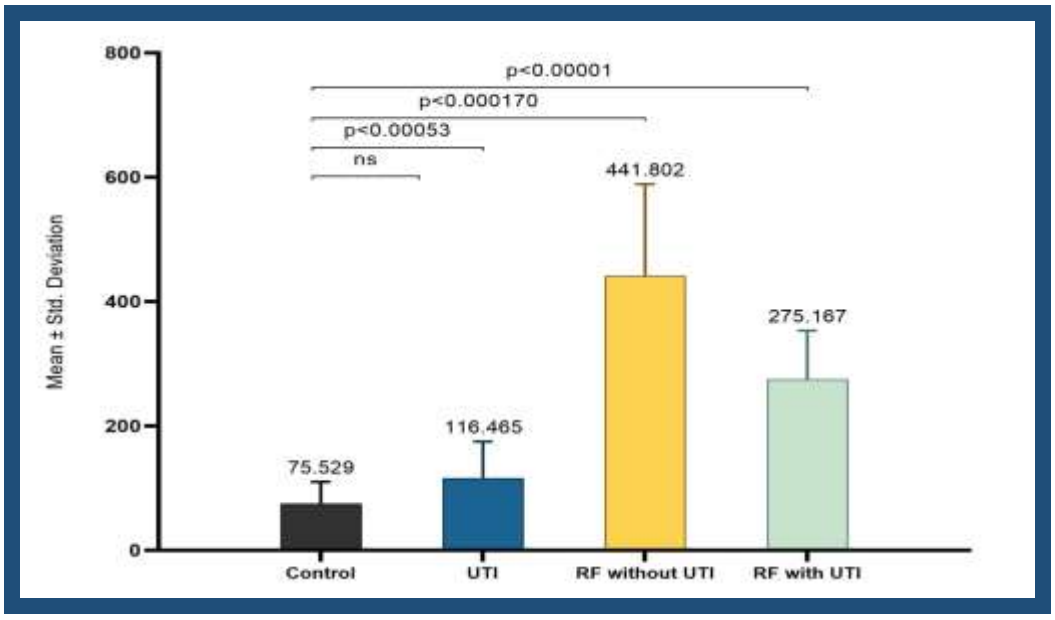

Figure 3-2: The Comparisons between study Groups According to the suPAR Parameter.

3.5: Distribution of FGF 23 between patients and controls

The FGF23 parameter showed a statistically significant difference across the study groups (p = 0.0003). The mean ± standard deviation of FGF23 levels was highest in the renal failure without UTI group (151.96 ± 130.33), followed by the renal failure with UTI group (115.16 ± 81.14), and the UTI group (33.41 ± 14.63). The control group had the lowest levels (25.19 ± 12.62). Overall, FGF23 levels were elevated in patients with renal failure—both with and without UTI—compared to those in the UTI and control groups.as shown in table 3-3

Table 3-5 : Comparisons between study Groups According to the FGF23 Parameter:

Groups	Mean	Std. Deviation	P. value
Control	25.185	12.619	0.0003
UTI	33.412	14.633	
RF without UTI	151.958	130.333	
RF with UTI	115.162	81.140	
ANOVA and Kruskal-Wallis tests *. The mean difference is significant at the 0.05 level. **. The mean difference is significant at the 0.01 level.			

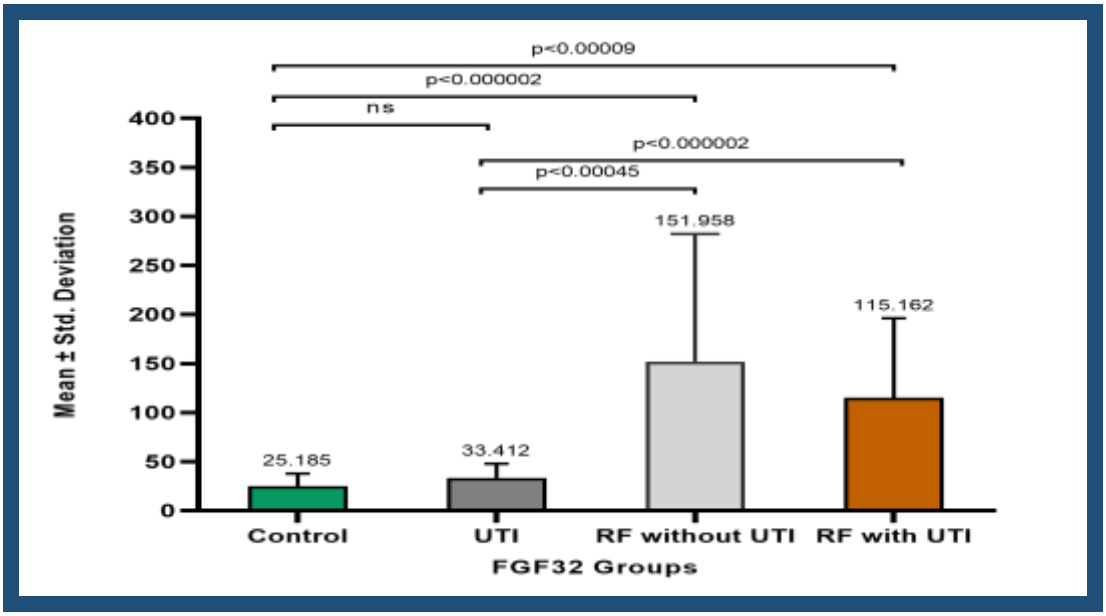

Figure 3-3: The Comparisons between study Groups According to the FGF23 Parameter.

3.6. Distribution of hematological and biochemical parameters between RF patient and the control

Several biochemical and hematological markers were compared among three groups: Control, Renal Failure (RF) without Urinary Tract Infection (UTI), and RF with UTI. This findings show significant differences ($p < 0.05$) in most measures across the groups, except serum iron (IRON), which did not demonstrate a statistically significant difference ($p = 0.5767$). Both RF groups, with and without UTI, exhibited notably elevated levels of serum phosphate (S. PO4), blood urea (B. UREA), serum creatinine (S. CREATININE), unsaturated iron-binding capacity (UIBC), serum ferritin, and parathyroid hormone (PTH) compared to the Control group. In contrast, levels of hemoglobin (HB), serum calcium (S.CA), and serum sodium (S.Na) were significantly lower in patients with RF.

Table 3-6: The comparison between study parameters in patients according to the research markers, stratified by patient groups and their controls

Parameters	Groups	Mean	Std. Deviation	P. value
S.PO4 (mmol/L)	Control	3.190	0.544	0.0006
	RF without UTI	6.303	2.377	
	RF with UTI	5.140	1.087	
S.CA (mg/dl)	Control	8.877	0.331	0.0133
	RF without UTI	8.413	0.732	
	RF with UTI	8.730	0.683	
S.Na (mmoI/L)	Control	145.167	8.918	0.0002
	RF without UTI	135.127	4.309	
	RF with UTI	132.980	14.376	
Hb (mg/dL)	Control	12.593	1.196	0.0001
	RF without UTI	8.577	1.525	
	RF with UTI	8.707	1.404	
B.UREA (mg/dL)	Control	27.400	10.170	0.0004
	RF without UTI	142.833	29.090	
	RF with UTI	139.933	44.754	
S.CREATININE (mg/dL)	Control	0.503	0.224	0.0003
	RF without UTI	8.177	2.670	
	RF with UTI	9.813	3.265	
IRON (mg/dL)	Control	77.700	31.322	0.5767
	RF without UTI	69.670	33.112	
	RF with UTI	69.623	37.890	
UIBC (mmol/L)	Control	185.367	49.841	0.0001
	RF without UTI	281.650	85.121	
	RF with UTI	260.200	82.913	
S .ferritin (mg/dl)	Control	68.100	28.271	0.0003
	RF without UTI	284.860	175.855	
	RF with UTI	227.117	119.457	
PTH (pg/ml)	Control	48.867	19.953	0.0005
	RF without UTI	376.933	312.205	
	RF with UTI	280.097	224.212	

ANOVA and Kruskal-Wallis tests *. The mean difference is significant at the 0.05 level. **. The mean difference is significant at the 0.01 level.

3.7. Correlation Coefficient Among study Parameters of RF Patients:

The correlation coefficients among research parameter and age in patients with renal failure (RF).The findings indicate substantial positive relationships between FGF23 and CFH ($r = 0.768$, $p < 0.001$) as well as between FGF23 and suPAR ($r = 0.674$, $p < 0.001$). CFH and suPAR have a robust correlation ($r = 0.670$, $p < 0.001$). Nevertheless, age exhibited no significant correlations with any of the factors ($p > 0.05$ for all comparisons).

Table 3-7: Correlation Coefficient Among Research Parameters (FGF 23, CFH, suPAR) of RF Patients

Parameters	Value	CFH	suPAR	Age
FGF23	**r. value**	.768**	.674**	.102
	p. value	.000	.000	.437
CFH	**r. value**	1.000	.670**	-.033
	p. value		.000	.801
suPAR	**r. value**		1.000	-.023
	p. value			.863
Age	**r. value**			1.000
	p. value			

3.8. Correlation Coefficient Among FGF 23 and Another Parameter of RF Patients

Levels of fibroblast growth factor 23 (FGF-23) were examined for possible relationships with other biochemical and hematological markers. The findings demonstrated a statistically significant positive correlation between FGF-23 and serum phosphorus levels ($r = 0.342$, $p = 0.008$), suggesting that elevated FGF-23 concentrations corresponded with heightened phosphorus levels. No more significant connections were detected between FGF-23 and the other parameters.

FGF-23 exhibited no significant correlation with parathyroid hormone (PTH) (r = -0.038, p = 0.774), iron (r = 0.157, p = 0.230), serum creatinine (r = 0.033, p = 0.802), blood urea (r = 0.222, p = 0.089), hemoglobin (r = -0.091, p = 0.490), ferritin (r = 0.069, p = 0.599), sodium (r = 0.196, p = 0.133), unsaturated iron-binding capacity (UIBC) (r = 0.039, p = 0.768), or calcium levels (r = -0.111, p = 0.398).

Table3-8 : correlation between FGF23 and another parameter :

Fibroblast growth factor 23 (pg/ml)	**Patient**	
	r	**p**
Parathyroid hormone (pg/ml)	-.038	.774
Iron (mg/dL)	.157	.230
Serum Creatinine (mg/dL)	.033	.802
Blood Urea (mg/dL)	.222	.089
Hemoglobin (mg/dL)	-.091	.490
Ferrtin (mg/dl)	.069	.599
phosphorus (mmol/L)	.342**	.008
Sodium (mmoI/L)	.196	.133
UIBC (mmol/L)	.039	.768
Calcium (mg/dl)	-.111	.398
-Pearson and Spearman's correlations . *. Correlation is significant at the 0.05 level. **. Correlation is significant at the 0.01 level.		

3.9. Receiver Operating Characteristic (ROC Test) in renal failure patients:

The diagnostic performance of immunological markers (FGF23, CFH, suPAR) in patients with renal failure (RF), FGF23 demonstrated the highest predictive accuracy (AUC: 97.0%, sensitivity: 90.0%, specificity: 96.7%, accuracy: 96.7%), closely followed by CFH (AUC: 96.8%, sensitivity: 90.0%, specificity: 96.7%),suPAR showed marginally diminished, although still strong performance (AUC: 93.6%, sensitivity: 88.3%, specificity: 83.3%). All markers exhibited statistically significant predictive capability ($p < 0.01$) and elevated positive/negative predictive values (PPV: 85.7–96.7%; NPV: 96.7–100%), with suPAR attaining an impeccable NPV (100%). The optimal diagnostic thresholds were FGF23: 49.5, CFH: 69.7, and suPAR: 104.4, corroborated by tight confidence intervals (Lower Bound ≥0.889, Upper Bound ≥0.982). These findings highlight FGF23 and CFH as exceptionally dependable biomarkers for RF diagnosis. As shown in table 3.9.

Table 3-9 : Receiver Operating Characteristic analysis shows the sensitivity, specificity and Cut off point for FGF23 , CFH and suPAR according to the renal faluir patients. .

Metrics		Renal failure		
		FGF23	CFH	suPAR
Std. Error		0.016	0.018	0.024
P value		0.004	0.005	0.002
Asymptotic 95% Confidence Interval	Lower Bound	0.939	0.933	0.889
	Upper Bound	1.000	1.000	0.982
Cutoff Point		49.507	69.677	104.373
Area Under Curve (AUC)		97.000%	96.833%	93.556%
Sensitivity		90.000%	90.000%	88.333%
Specificity		96.567%	96.645%	83.333%
Accuracy		96.755%	96.667%	91.667%
Positive Predictive Value		96.460%	96.543%	85.714%
Negative Predictive Value		96.648%	96.722%	100.000%

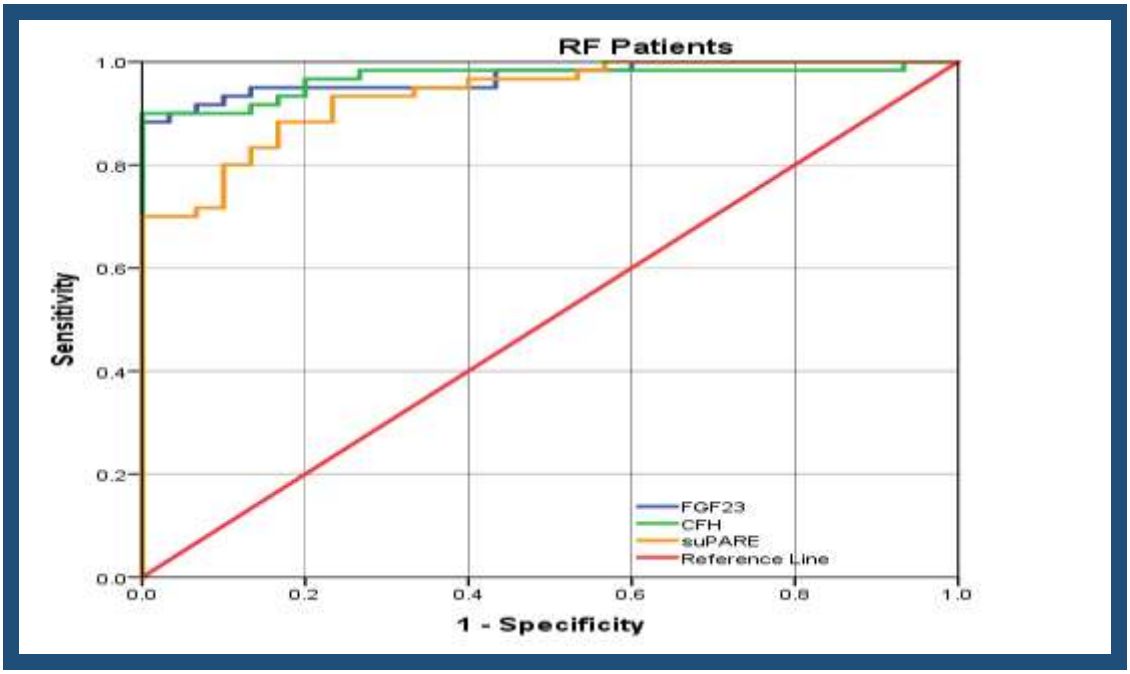

Figure 3-4: ROC analysis illustrating the sensitivity and specificity values for FGF23, CFH, and suPAR of renal failure patients (RF Patients).

3.10. Receiver Operating Characteristic (ROC Test) in UTI :

The diagnostic efficacy of immunological markers (FGF23, CFH, suPAR) is identifying urinary tract infections (UTI) , suPAR exhibited the highest predictive capability (AUC: 75.0%, sensitivity: 73.3%, specificity: 76.7%, accuracy: 75.0%), succeeded by CFH (AUC: 72.7%, sensitivity: 63.3%, specificity: 73.3%) and FGF23 (AUC: 64.2%, sensitivity: 96.7%, specificity: 33.3%). FGF23 had excellent sensitivity (96.7%) and negative predictive value (NPV: 90.9%), but its low specificity (33.3%) constrains diagnostic reliability. These data indicate that suPAR and CFH may more effectively differentiate UTI cases than FGF23, which demonstrates a high rate of false positives.as shown in table 3.1.

Table 3.10 : ROC analysis shows the sensitivity, specificity and Cut off point for FGF23 , CFH and suPAR according to the UTI patients

Metrics		UTI		
		FGF23	CFH	suPAR
Std. Error		0.071	0.065	0.065
Asymptotic Sig.		0.058	0.003	0.001
Asymptotic 95% Confidence Interval	Lower Bound	0.502	0.600	0.623
	Upper Bound	0.782	0.853	0.877
Cutoff Point		17.153	49.847	86.797
Area Under Curve (AUC)		64.222%	72.667%	75.000%
Sensitivity		96.667%	63.333%	73.333%
Specificity		33.333%	73.333%	76.667%
Accuracy		65.000%	68.333%	75.000%
Positive Predictive Value		59.184%	70.370%	75.862%
Negative Predictive Value		90.909%	66.667%	74.194%

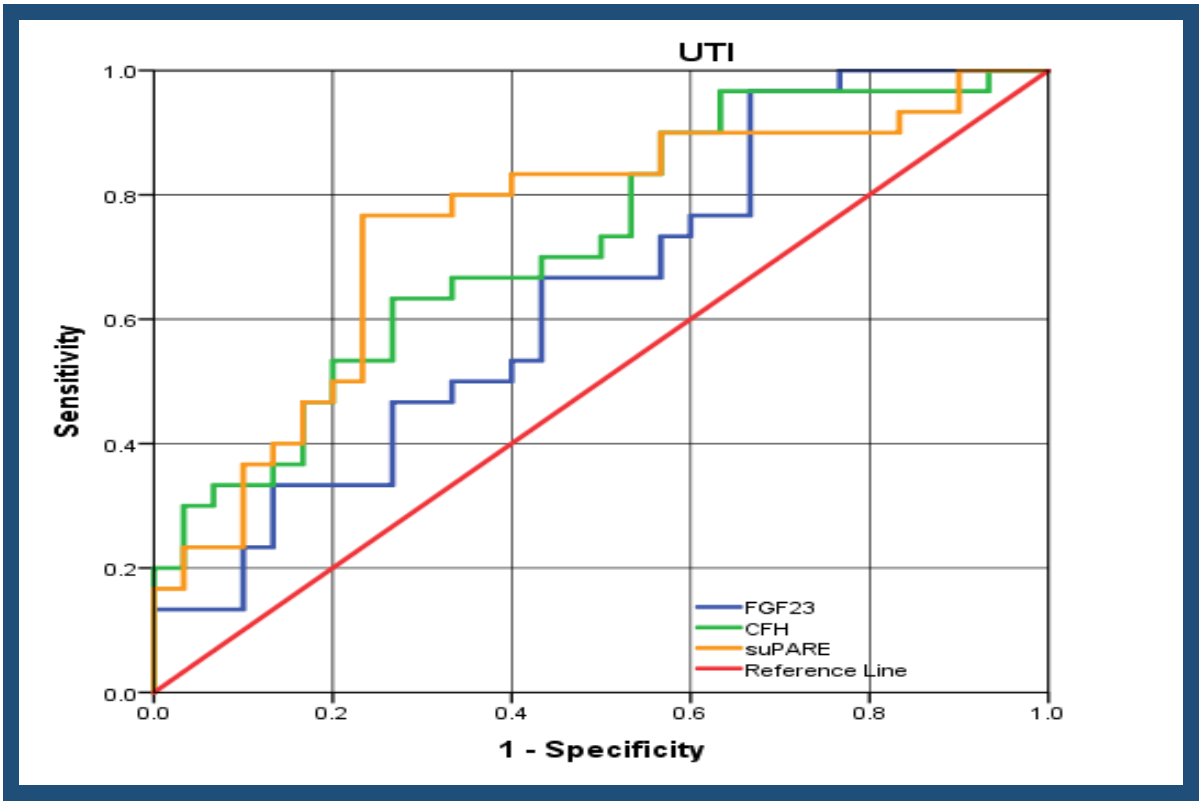

Figure 3-5: ROC analysis illustrating the sensitivity and specificity values for FGF23, CFH, and suPAR of UTI patients .

Chapter Four
Discussions

4. Discussion

4.1. Demographic Characteristics of Study Groups

This research demonstrated in table 3-1 that females are far more susceptible to urinary tract infections than males. The youngest age group (<40 years) exhibited the highest susceptibility to UTIs, closely followed by older participants (≥60 years). Notably, while middle-aged adults (40–59 years) had a somewhat lower prevalence of UTIs, they demonstrated a higher proportion of risk factors linked with UTIs.

When compared with previous studies, these findings presented here closely align with established research(Zhan *et al.*, 2024; Timm, Russell and Hultgren, 2025)**,** emphasizing that women have greater susceptibility to UTIs primarily due to anatomical differences, notably their shorter urethra, facilitating bacterial access to the bladder**.** In the present study, it was observed that males predominate in RF without UTI cases, whereas females predominate in RF with UTI cases. This agrees with(Yamashita *et al.*, 2022), indicating that men are more susceptible to renal failure from systemic illnesses such as hypertension or diabetes. In contrast, women are more vulnerable to urinary tract infections due to anatomical features. but disagreement with (Majeed and Aljanaby, 2019)**.** A study in Al-Najaf City, Iraq, revealed that among 120 patients with UTIs, the predominant age group was 51–60 years, accounting for 40% of the total cases. This cohort comprised 22 patients without kidney disease (WKD) and 26 patients with chronic kidney disease (CKD) out of 48 participants.

Also in this study, RF without UTI cases, younger patients (<40 years) are more prevalent (40%), while elderly patients (≥60 years) dominate RF with UTI cases (33.33%) agree with (Dimitrijevic *et al.*, 2021)**.**That showed increased risk of UTIs among elderly individuals, which is attributable to their weakened immune

systems and comorbidities agreed with (Dimitrijevic *et al.*, 2021; Esposito *et al.*, 2024) A thorough comprehension of sex and age characteristics is essential for formulating targeted prevention and treatment strategies for renal failure**.**

4.2: Distribution of Pathogens among patient groups :

Table 2 illustrates the prevalence of several pathogens in urinary tract infections (UTIs) and their occurrence in instances of renal failure (RF) associated with UTIs. Escherichia coli is the predominant pathogen in urinary tract infections, representing 50% of cases in the supplied data

When compared with previous studies our result agreed with(Manhal, Mohammed and Ali, 2012), *E. coli* was the predominant pathogen in UTI in renal failure patients. (Popovici, 2022)Research consistently identifies *E. coli* as the predominant cause of urinary tract infections, with incidence rates between 41% and 83% across different contexts**.** (Choi *et al.*, 2022)The analysis identifies Escherichia coli as the primary causative agent of urinary tract infections (UTIs), underscoring its substantial impact on disease prevalence and the drug susceptibility patterns that influence human health and society.(Niu *et al.*, 2023)The predominance of *E. coli* is due to its capacity to effectively colonize the urinary system, aided by certain virulence factors.

Following *E. coli*, other significant pathogens include *K. pneumoniae* is the second most commonly isolated pathogen in urinary tract infections, after *E. coli*, in both community and hospital environments. In patients with chronic kidney disease, *K. pneumoniae* was responsible for 32.60% of UTI cases, indicating its substantial role in this population(Cristea *et al.*, 2017)**.** Additional infections, including *Proteus mirabilis*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*, contribute to the

situation, with prevalence rates differing according to patient demographics and underlying health conditions (Tarchouna *et al.*, 2013)

4.3. Distribution of CFH between patient and control groups

In table 3-4 CFH levels differed significantly (P=0.0006), with renal failure (RF) groups showing markedly elevated values (291.40 ± 199.58 without UTI; 222.48 ± 189.98 with UTI) compared to controls (42.49 ± 14.97) and UTI-only patients (59.19 ± 22.72). High variability in RF groups, though reduced CFH in RF+UTI vs. RF alone hints at UTI moderation, while CFH elevation aligns with renal pathology.

Our study agreed with (Nagamachi *et al.*, 2014) who showed that the study found that urinary concentrations of properdin (P), factor H (fH), and membrane attack complex (MAC) were significantly higher in patients with renal diseases compared to normal controls.

Also, (Wen *et al.*, 2019) showed Urinary CFH, membrane attack complex and serum CFH were increased in both IgAN and CKD patients compared with healthy controls.

4.4. Distribution of suPAR between patient and control

In table 3-5 suPAR levels exhibited substantial variation among groups and showed significant differences (p-value= 0.0002 **),**Controls exhibited the lowest levels followed by UTI patients. Patients with renal failure (RF) without urinary tract infection (UTI) had the highest levels , whereas those with RF with UTI demonstrated lower yet elevated values.

The result of this study agreed with (Valaperta *et al.*, 2024) who found a significant correlation between elevated plasma suPAR levels and a decline in estimated glomerular filtration rate (eGFR) (P=0.0001), indicating that higher suPAR levels may be associated with worsening kidney function .(Skalec *et al.*,

2022) who demonstrated that suPAR levels are markedly increased in septic patients with acute kidney injury (AKI) relative to a control group devoid of sepsis and renal failure (13.01 ng/mL vs. 4.05 ng/mL, $p < 0.001$). (Iversen *et al.*, 2020)A reported that elevated suPAR levels at hospital admission correlated with a heightened risk of developing both chronic and acute kidney diseases, increased suPAR levels have been associated with the advancement of chronic kidney disease (CKD),underscoring its potential as a biomarker for renal pathology.

(İşsever and Dheir, 2023) The research indicated that suPAR levels were markedly elevated in patients with glomerulonephritis relative to healthy subjects, with mean levels of 166.06 ± 127.66 pg/ml in patients compared to 119.67 ± 70.53 pg/ml in the control group ($p = 0.001$). However,(Ni *et al.*, 2016)showed suPAR is identified as a biomarker of immunological activation and inflammation, with increased levels found in numerous bacterial infections. A meta-analysis demonstrated suPAR's effectiveness in diagnosing and prognosticating bacterial infections, revealing a pooled sensitivity of 0.73 and specificity of 0.79 for infection diagnosis**.**

4.5. Distribution of FGF 23 between patient and control groups

In this study, table 3-3 there were significant differences (p-value = 0.0002) when compared between study groups with control group. mean of FGF23 levels are minimal in the control group and maximal in the renal failure (RF) group without urinary tract infection (UTI). This finding was similar to the study conducted by (Anbarasan and Khanna, 2019; Ahmed and Younis, 2023)**,** who showed statically significant ($P<0.05$) and that the mean serum FGF-23 level in CKD patients was considerably elevated at 730.7±492.7 pg/ml, in contrast to 39.49±12.47 pg/ml in the control group. This indicates a notable elevation in FGF-23 levels among RF patients.

In the present study, it was observed elevation in the average concentration of FGF23 in individuals with renal failure compared with the control group. These results were in line with the result (Ibrahim and Rashed, 2009; Rodríguez-Ortiz *et al.*, 2020) which showed FGF23 levels increased in renal failure due to the impaired regulation of phosphorus excretion and calcitriol levels**.** Increased concentrations of Fibroblast Growth Factor 23 (FGF23) in renal failure with out of urinary tract infections (UTIs), as compared to renal failure accompanied by UTI that can be ascribed to many factors associated with the pathophysiology of chronic kidney disease (CKD) and the function of FGF23 in phosphate metabolism. FGF23 is a hormone chiefly responsible for maintaining phosphate homeostasis, with its levels greatly elevated in chronic kidney disease (CKD) due to diminished renal clearance and heightened synthesis by osteocytes. The lack of UTI may affect the inflammatory condition and phosphate management in these patients, potentially resulting in elevated FGF23 levels**.**

4.6. Distribution of hematological and biochemical parameters between RF patient and the control

The study highlights significant differences in biochemical, hematologic, and hormonal parameters among study groups table 3-6, both RF groups exhibited significantly raised levels of urea and creatinine, with the RF+UTI group demonstrating higher creatinine levels. This study agreed with (Iqbaal *et al.*, 2024) who showed that Concentration of urea and creatinine was higher in males than in females in both groups. Values of post-dialysis urea and creatinine were significantly ($p < 0.01$) lower than their pre-dialysis values but were still significantly higher than the health group, as urea 27 ± 4.7 mg/dL and 1.5 ± 0.07

mg/dL creatinine. Our results agreed with (R *et al.*, 2017; Vanholder, Gryp and Glorieux, 2018; Laville *et al.*, 2022) who investigated serum creatinine and urea concentrations in 50 individuals with renal failure before and following hemodialysis, demonstrating considerably elevated levels of both indicators before the procedure, which markedly diminished post-dialysis. This demonstrates the efficacy of hemodialysis in diminishing harmful metabolic byproducts in individuals with renal failure. Increased urea concentrations are linked to renal impairment and regarded as both a direct and indirect uremic toxin, contributing to cardiovascular disease and systemic inflammation. Urea plays a role in systemic inflammation and endothelial dysfunction, which are pivotal in the advancement of cardiovascular disease in patients with chronic kidney disease.

In this study table 3-6, showed electrolyte abnormalities in renal faluire patients this agreed with (Yaxley and Yaxley, 2023). The present investigation of electrolyte abnormalities comprised hyperphosphatemia, presumably attributable to infection-induced fluid changes indicated that phosphorus levels were significantly elevated in patients compared to apparently healthy controls, whereas calcium and sodium levels were significantly reduced in patients relative to apparently healthy controls this finding aligns with(Inagi *et al.*, 2008). assert that declining kidney function impairs phosphate excretion, resulting in retention and increased serum concentrations. This understanding that hyperphosphatemia is a characteristic of chronic kidney disease (CKD) resulting from diminished phosphate excretion.

The disparity between RF in UTI and non-UTI groups may illustrate the complex relationship between inflammation and mineral metabolism in individuals with kidney disease, as articulated by (Matsui *et al.*, 2018).

As mentioned in this study the blood concentration of Hb was significantly lower in patients compared to the control group. Anemia in RF groups against controls indicated chronic inflammation, corroborated by increased ferritin and UIBC levels, and this finding aligns with (Agoro *et al.*, 2018; Micarelli *et al.*, 2020). Who demonstrated that severe anemia is a common effect in chronic kidney disease (CKD) and constitutes a risk factor for cardiovascular disease and heart failure in CKD patients. Renal anemia is caused by multiple factors, including reduced erythropoietin production, iron insufficiency, and inflammation. Previous investigations have revealed no relationship between elevated FGF23 levels and renal anemia, based on clinical research. An observational research involving 53 CKD patients demonstrated a negative correlation between elevated FGF23 levels and hemoglobin levels. (Portolés *et al.*, 2021) The study showed that condition of anemia found in both renal failure cohorts is likely attributable to several factors: Reduced erythropoietin (EPO) synthesis by the dysfunctional kidneys, which constitutes the principal mechanism of anemia in chronic kidney disease (CKD).(Ganz *et al.*, 2020) found that reduced lifetime of red blood cells attributable to uremic toxins, impaired iron consumption despite sufficient reserves (functional iron insufficiency) and chronic inflammation, especially in individuals with associated urinary tract infection.

In addition,in our study, a secondary hyperparathyroidism (PTH: in RF vs. RF+UTI) indicates reduced PTH in RF+UTI, potentially associated with inflammatory suppression. The significantly raised PTH values in both renal failure cohorts indicate secondary hyperparathyroidism (SHPT), a recognized consequence of chronic kidney disease (CKD). This finding aligns with(Mahmood, 2023) who observed a notable elevation in parathyroid hormone (PTH) levels in patients with end-stage renal failure (ESRD) (275.16 ± 224.03 pg/ml) in contrast to healthy

subjects (44.42 ± 19.26 pg/ml). Furthermore, phosphate levels were markedly elevated in ESRD patients (1.52 ± 0.53 mmol/L) in comparison to the healthy cohort (1.04 ± 0.15 mmol/L) . (Sajjad *et al.*, 2014) study comprised 50 patients with a mean age of 54.6 years, of whom 76% exhibited parathyroid hormone (PTH) levels exceeding the normal range. The mean serum concentrations of calcium, phosphate, and PTH were 8.32 mg/dl, 4.42 mg/dl, and 125.45 pg/ml, respectively, suggesting that although PTH levels were elevated, they remained within the acceptable reference range.

However, Patients with renal failure and urinary tract infections exhibit lower mean parathyroid hormone levels (280.097 pg/mL) than those without urinary tract infections (376.933 pg/mL), while both groups demonstrate considerably higher levels relative to controls, Several mechanisms may explain this finding(Naskar and Choi, 2024) Inflammatory modulation: Urinary tract infections stimulate the generation of inflammatory cytokines that may affect parathyroid hormone secretion. Studies indicate that inflammatory cytokines such as IL-1β, IL-6, and TNF-α can influence parathyroid gland activity and the secretion of parathyroid hormone (PTH). (Iamartino and Brandi, 2022) Modified calcium-sensing receptor sensitivity. Inflammatory conditions may alter the sensitivity of calcium-sensing receptors on parathyroid cells, influencing PTH secretion.. (Rivara *et al.*, 2018)Patients with urinary tract infections may undergo therapies that indirectly influence mineral metabolism and parathyroid hormone levels.

4.7.Correlation Coefficient Among Research Parameters of RF Patients

Positive Correlation coefficients among three biomarkers table 3-7, Fibroblast Growth Factor 23 (FGF23), Complement Factor H (CFH), and soluble urokinase-type Plasminogen Activator Receptor (suPAR)—and age in patients with renal failure.

FGF23 and CFH: The most significant connection is observed between FGF23 and CFH ($r = 0.768$, $p < 0.001$) (table 3-7) , our study agreed with (Chen *et al.*, 2021) showed a clear association between the dysregulation of mineral metabolism and the activation of the complement system. This association is significant since it connects two pathophysiological systems related to kidney disease progression. FGF23 is a hormone that plays a role in phosphate metabolism and is recognized to be higher in chronic kidney disease (CKD) and end-stage renal disease (ESRD). It is linked to mineral bone problems, cardiovascular disease, and inflammation. (Mendoza *et al.*, 2012; Singh *et al.*, 2014) reported that FGF23 has been demonstrated to correlate with inflammatory markers, including interleukin-6 (IL-6), C-reactive protein (CRP), and tumor necrosis factor-alpha (TNF-α) in patients with chronic kidney disease (CKD)**.** (Tahir *et al.*, 2022)The interaction between FGF23 and suPAR may be substantial, as both participate in inflammatory pathways and might potentially worsen kidney damage and cardiovascular risk in people with renal failure**.** the strong correlation between FGF23 and CFH suggestes potential noval cross-talk between dysregulated mineral balance and chronic inflammation /complement activation in renal faluire, both of which are known driveres of disease progression .

FGF23 and suPAR: A strong positive association ($r = 0.674$, $p < 0.001$) associates FGF23 with suPAR (table 3-7) , which agreed with (Liu *et al.*, 2022)**,** who indicated an interaction between phosphate regulation and inflammatory/immune activation mechanisms. Recent studies indicate that both markers independently predict negative renal outcomes, with FGF23 exhibiting a pooled relative risk of 1.21 and suPAR presenting a risk of 1.42 for the advancement of kidney disease**.** (Abinti *et al.*, 2024) who showed the correlation between FGF23 and inflammation may suggest a possible interaction with suPAR,

a marker of inflammation associated with the advancement of kidney disease. (Munoz Mendoza *et al.*, 2012) assumed that chronic inflammation in renal failure likely induces simultaneous elevations in all three biomarkers. Inflammatory cytokines can induce the synthesis of FGF23 **.**

CFH and suPAR: CFH exhibits a strong positive correlation with suPAR ($r = 0.670$, $p < 0.001$) (table 3-7) ,this agreed with (Wei *et al.*, 2011) underscoring possible associations between the regulation of the complement system and inflammatory cascades that lead to kidney injury **.** (Singh *et al.*, 2014) reported that suPAR is considered as a potential early biomarker for chronic kidney disease (CKD), with elevated levels signifying an increased risk of disease development**.** (Reisinager *et al.*, 2021; García, Aggarwal and Tang, 2023) showed the interaction between suPAR and the complement system, controlled by CFH, may affect renal disease. Increased suPAR levels may intensify inflammation, whereas CFH dysregulation could result in complement-mediated injury, thereby advancing renal failure development.

4.8. Correlation Coefficient Among FGF 23 and another parameter of RF Patients

Correlation coefficients between Fibroblast Growth Factor 23 (FGF23) and diverse biochemical markers in individuals with renal disease and the most important find in this correlation analysis is the statistically significant positive association between FGF23 and phosphorus ($r = 0.342$, $p = 0.008$). Although no correlation with other parameters that showed in table 3-8 .This correlation aligns with other research (Hussain *et al.*, 2023) A study investigated individuals with chronic kidney disease undergoing hemodialysis found a moderate association between FGF23 and phosphate ($r = 0.134$, $p = 0.354$) was found that lacked statistical significance .(Kawabata *et al.*, 2020) This correlation confirms the

biological association between FGF23 and phosphorus metabolism, elevated serum phosphorus levels induce FGF23 production in osteocytes, facilitating urinary phosphate excretion and inhibiting 1,25-dihydroxyvitamin D synthesis**.** In individuals with diminished renal function, this homeostatic mechanism is impaired, leading to increased levels of FGF23 and phosphorus, hence elucidating the observed positive interaction**.** Our study disagrees with (Zeng *et al.*, 2023) who showed that FGF23 had positive relationships with PTH ($r = 0.25$, $p = 0.010$) and calcium ($r = 0.27$, $p = 0.005$) in patients undergoing maintenance hemodialysis; however, the multivariate analysis did not indicate a significant independent link with serum phosphorus ($p = 0.990$).

4.9. Receiver Operating Characteristic (ROC Test) in renal failure

ROC analysis for immunological markers (suPAR,CFH and FGF 23) in renal faluire groups showed All three indicators exhibit strong predictive power, with AUCs over 90%, signifying an exceptional capacity to differentiate between individuals with and without renal failure. The statistically significant p-values (Asymptotic Sig.: 0.004–0.002) validate that all AUCs surpass random chance (AUC = 0.5) with significance in table 3-9 .

This corresponds with the increasing interest in utilizing biomarkers for early diagnosis and risk assessment in renal illnesses, as emphasized in previous studies (A.Kamel *et al.*, 2022) who showed Sensitivity 85% Specificity 90% PPV 94.4% NPP 75% Accuracy 80% , as for the cutoff point for FGF23's ability to accurately predict kidney disease, and an AUROC of 0.837 and a P value of 0.0001.(Zhang and Qin, 2023) The serum FGF23 had exceptional predictive capacity for acute kidney injury, with an AUC of 0.9, with 100% sensitivity and 97.1% specificity.

The cutoff value of 49.507 pg/mL delineated in table 3-9 is notably similar with results from several research (Chen *et al.*, 2022)determined an optimal FGF23 threshold of approximately 50 pg/mL for forecasting rapid renal function deterioration, with the authors observing that "The results indicate that elevated FGF-23 levels (cut-off value: 32-50 pg/mL) were correlated with an increased likelihood of rapid kidney function decline.

In 2019, (Fayed *et al.*, 2019) examined FGF23 as a prognostic indicator of death in patients with renal illness, identifying a cutoff value of 48 pg/mL, with a sensitivity of 77.5% and a specificity of over 90%, confirming a high specificity of 96.567%**.** (Zhou, Liu and Yang, 2024) FGF23 has been recognized for its involvement in phosphate metabolism and has been linked to the progression of chronic kidney disease (CKD). (Vázquez-Sánchez *et al.*, 2021) FGF23 is recognized as a significant biomarker for diagnosing and prognosticating mineral bone diseases **,** while suPAR serves as a universal and widely applicable prognostic biomarker for chronic inflammation (Pu and Xu, 2024)**.**

In this study, Complement Factor H (CFH) has been identified as a significant biomarker for predicting renal failure, as seen by the 96.833% AUC presented in table 3-9, agree with (Kesarwani *et al.*, 2024) established that "Complement factor H (CFH) is pivotal in regulating the complement alternative pathway," and genetic differences in CFH are significantly correlated with the course of renal illness.(Bonomo *et al.*, 2014) this research indicated that "CFH was correlated with frequently reported causes of end-stage kidney disease in the population" and displayed outstanding predictive attributes, with AUC values continuously above 0.95.(Xu *et al.*, 2025) further confirmed CFH's predictive capacity for renal injury, indicating that "genetic variations in serum complement factor H (CFH) affect complement activation and correlate with the advancement of

kidney disease," however , in this study suPAR was a high value of AUC this agree with (Huang *et al.*, 2023). The meta-analysis revealed that the overall sensitivity of suPAR for predicting AKI was 0.77 (95% CI 0.67-0.84), while the specificity was 0.64 (95% CI 0.53-0.75). The diagnostic odds ratio was 6 (95% CI 3-10), the pooled positive likelihood ratio was 2.2 (95% CI 1.6-2.9), and the pooled negative likelihood ratio was 0.36 (95% CI 0.26-0.52). Additionally, the area under the summary receiver-operating characteristic (SROC) curve was 0.77 (95% CI 0.12-0.99).

The sensitivity (88.333%) and specificity (83.333%) results align with the findings of (Hayek *et al.*, 2017) who reported that "suPAR strongly predicts outcomes and incident chronic kidney disease in patients with cardiovascular disease" with comparable diagnostic metrics.

The elevated AUC values indicate that these markers are dependable for forecasting renal failure, which is essential for early intervention and management measures (Ramspek *et al.*, 2021; Samanta, Bandyopadhyay and Samanta, 2023)

The standard errors (suPAR: 0.008, FGF23: 0.020, CFH: 0.031) indicate high precision, particularly for suPAR. As noted in research: "ROC curve analysis can be used to evaluate if a novel biomarker might be useful in the diagnosis of a certain condition (Roumeliotis *et al.*, 2024).

4.10. Receiver Operating Characteristic (ROC Test) in UTI

A study evaluated immunological markers (FGF23, CFH, suPAR) for diagnostic performance UTI using metrics like AUC, sensitivity, specificity, accuracy, and predictive values. suPAR demonstrates the strongest overall performance among the three biomarkers with several advantages: highest AUC (75.000%) ,most statistically significant (p=0.001) , best balance between

sensitivity (73.333%) and specificity (76.667%) and highest accuracy (75.000%) that showed in table 3-10.

Previous research supports these findings, (Reisinager *et al.*, 2021) indicated that suPAR levels are markedly increased during active urinary tract infections and correlate with the severity of the infection.(Zhang *et al.*, 2009) Research has shown that complement factor H (CFH) is significant not only in bladder cancers but also potentially in urinary tract infections (UTIs). Certain research indicate that quantifying CFH levels in urine may aid in evaluating inflammation and could signify the existence of infections. CFH's inhibitory role in the complement alternative pathway indicates its participation in modulating the immunological response to infections**.**

(Raitanen *et al.*, 2001)The thorough examination of urine for multiple biomarkers, including CFH and its associated proteins, substantiates the idea that these components can act as indications of renal dysfunction and damage due to urinary tract infections. This enhances the value of CFH in clinical settings, especially in differentiating various etiologies of urine symptoms and successfully monitoring disease activity. In this study, FGF 23 poor prognostic marker in UTI, which agrees with Numerous studies(Bienaimé *et al.*, 2017) indicate elevated levels of FGF23 in urinary samples may signify a poor prognosis in patients with diverse infections, particularly those involving the urinary tract. Specifically, heightened FGF23 concentrations have been linked to an augmented risk of hospitalization due to infection, demonstrating its potential utility in identifying patients at increased risk of severe complications associated with UTIs**.**

Conclusions

&

Recommendations

Conclusions :

1-Immunological markers (**FGF23, CFH, and suPAR)** were significantly elevated in RF groups (with/without UTI) compared with controls and UTI.

2- FGF23 and CFH exhibited strong mutual correlations with suPAR, highlighting their interconnected roles in renal pathophysiology. Also, FGF23 correlated positively with serum phosphorus, aligning with its known regulatory role in mineral metabolism.

3-FGF23 and CFH demonstrated exceptional accuracy, specificity, supporting their role as strong biomarkers for RF diagnosis, potentially enabling early detection and monitoring.

4-suPAR showed superior discriminative power (AUC, specificity) compared to FGF23 and CFH, which had lower specificity, thus, it can be used as a biomarker for the diagnosis of UTIs.

Recommendations :

1-Studying of another type of microbial agent, like viral and fungal infections, among hemodialysis patients.

2- Following-up for long time of immunological markers(FGF 23,CFH,and suPAR) for hemodialysis patients .

3-Studying gene polymorphism of these markers and their relationship to renal failure disease.

4-Studying and measurement concentration of these immunological markers before and after dialysis.

5-Studying correlation of another parameters with these immunological markers in renal failure patients.

References

A.Kamel, A., S.ElHamshary, A., R.AbdelGwad, E. and A.Elsayed, R. (2022) 'Serum Fibroblast Growth Factor 23 as an Early Biomarker for Detection of Renal Osteodystrophy and Progression of Chronic Kidney Disease in Children', *Benha Journal of Applied Sciences*, 7(6), pp. 109–117. Available at: https://doi.org/10.21608/bjas.2022.252249.

Abbasi, M.A., Chertow, G.M. and Hall, Y.N. (2010) 'End-stage renal disease.', *BMJ clinical evidence*, 2010.

Abinti, M., Vettoretti, S., Armelloni, S., Molinari, P. and Castellano, G. (2024) 'Associations of Intact and C-Terminal FGF23 with Inflammatory Markers in Older Patients Affected by Advanced Chronic Kidney Disease', *Stomatology*, 13(13), p. 3967. Available at: https://doi.org/10.3390/jcm13133967.

Agoro, R., Montagna, A., Goetz, R., Aligbe, O., Singh, G., Coe, L.M., Mohammadi, M., Rivella, S. and Sitara, D. (2018) 'Inhibition of fibroblast growth factor 23 (FGF23) signaling rescues renal anemia.', *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*, 32(7), pp. 3752–3764. Available at: https://doi.org/10.1096/fj.201700667R.

A**hmed, H.M. and Younis, N.T.** (2023) 'Biochemical Study on Fibroblast growth factor 23(FGF23) and its relation with Chronic Kidney Disease', *Research journal of pharmacy and technology*, pp. 115–118. Available at: https://doi.org/10.52711/0974-360x.2023.00021.

Al-Badr, A. and Al-Shaikh, G. (2013) 'Recurrent Urinary Tract Infections Management in Women: A review.', *Sultan Qaboos University medical journal*, 13(3), pp. 359–367. Available at: https://doi.org/10.12816/0003256.

Alhazmi, A.I., Alghamdi, A.H.A., Alzahrani, K.A.M., Alzahrani, R.A.A.B., Al Ghamdı, I.A.I. and Alzahrani, M.K.B. (2023) 'Leading Causes of Chronic Kidney Disease Among Dialysis Patients in Al-Baha Region, Saudi Arabia.', *Cureus*, 15(11), p. e49439. Available at: https://doi.org/10.7759/cureus.49439.

Aljawadi, M.H., Babaeer, A.A., Alghamdi, A.S., Alhammad, A.M., Almuqbil, M.S. and Alonazi, K.F. (2024) 'Quality of life tools among patients on dialysis: A systematic review', *Saudi Pharmaceutical Journal*, 32(3), p. 101958. Available at: https://doi.org/10.1016/j.jsps.2024.101958.

Alkhaqani, A.L. (2022) 'Complications of Chronic Kidney Disease: Narrative Review', *Al-Rafidain Journal of Medical Sciences*, 2, pp. 107–114. Available at:

https://doi.org/10.54133/ajms.v2i.68.

Amorim, R.G., Guedes, G. da S., Vasconcelos, S.M. de L. and Santos, J.C. de F. (2019) 'Kidney disease in diabetes mellitus: Cross-linking between hyperglycemia, redox imbalance and inflammation', *Arquivos Brasileiros de Cardiologia*, 112(5), pp. 577–587. Available at: https://doi.org/10.5935/abc.20190077.

Abbott Ireland Diagnostics Division (2022) Urea Nitrogen2 assay. Available at: https://www.accessdata.fda.gov/cdrh_docs/pdf20/K203771.pdf (Accessed: 10 September 2025).

Anandh, U., Mandavkar, P., Das, B. and Rao, S. (2017) 'Fibroblast growth factor-23 levels in maintenance hemodialysis patients in India.', *Indian journal of nephrology*, 27(1), pp. 9–12. Available at: https://doi.org/10.4103/0971-4065.177137.

Anbarasan, P. and Khanna, V. (2019) 'Serum fibroblast growth factor 23 levels in chronic kidney disease and its correlation with bio-chemical parameters in chronic kidney disease: a cross sectional comparative study', *International Journal of Advances in Medicine*, 6(4), p. 1101. Available at: https://doi.org/10.18203/2349-3933.ijam20192587.

Angeletti, A., Zappulo, F., Donadei, C., Cappuccilli, M., Di Certo, G., Conte, D., Comai, G., Donati, G. and La Manna, G. (2020) 'Immunological effects of a single hemodialysis treatment', *Medicina*, 56(2), p. 71.

Aniba, R., Dihmane, A., Raqraq, H., Ressmi, A., Nayme, K., Timinouni, M. and Barguigua, A. (2024) 'Exploring staphylococcus in urinary tract infections: A systematic review and meta-analysis on the epidemiology, antibiotic resistance and biofilm formation', *Diagnostic Microbiology and Infectious Disease*, 110(4), p. 116470. Available at: https://doi.org/https://doi.org/10.1016/j.diagmicrobio.2024.116470.

de Arriba, G., Avila, G.G., Guinea, M.T., Alia, I.M., Herruzo, J.A., Ruiz, B.R., Tejeiro, R.D., Rubio, M.E.L., Poyatos, C.V. and Roldán, C.G. (2021) 'Mortality of hemodialysis patients is associated with their clinical situation at the start of treatment', *Nefrologia*, 41(4), pp. 461–466. Available at: https://doi.org/10.1016/j.nefroe.2021.10.006.

Bahramian, A., Khoshnood, S., Hashemi, N., Moradi, M., Karimi-Yazdi, M., Jalallou, N. and Saki, M. (2021) 'Identification of metallo-β-lactamases and AmpC production among Escherichia coli strains isolated from hemodialysis

patients with urinary tract infection', *Molecular Biology Reports*, 48(12), pp. 7883–7892. Available at: https://doi.org/10.1007/s11033-021-06814-y.

B**anaga, A.S.I., Mohammed, E.B., Siddig, R.M., Salama, D.E., Elbashir, S.B., Khojali, M.O., Babiker, R.A., Elmusharaf, K. and Homeida, M.M.** (2015) 'Causes of end stage renal failure among haemodialysis patients in Khartoum State/Sudan', *BMC Research Notes*, 8(1), pp. 1–7. Available at: https://doi.org/10.1186/s13104-015-1509-x.

Bär, L., Stournaras, C., Lang, F. and Föller, M. (2019) 'Regulation of fibroblast growth factor 23 (FGF23) in health and disease', *FEBS Letters*, 593(15), pp. 1879–1900. Available at: https://doi.org/10.1002/1873-3468.13494.

Bayülgen, M.Y. and Gün, M. (2022) 'Effect of Complementary and Integrative Treatments on Fatigue Symptoms in Hemodialysis Patients: A Systematic Review', *Holistic Nursing Practice*, 36(1). Available at: https://journals.lww.com/hnpjournal/fulltext/2022/01000/effect_of_complementary_and_integrative_treatments.5.aspx.

Bazydlo, L., Needham, M. and Harris, N. (2014) 'Calcium, Magnesium, and Phosphate', *Laboratory Medicine*, 45, pp. e44–e50. Available at: https://doi.org/10.1309/LMGLMZ8CIYMFNOGX.

BT Laboratory (2024) Human CFH ELISA Kit (Cat. No.: E0324Hu); Human suPAR ELISA Kit (Cat. No E3759Hu):HumanFGF23 (cat. No: E0059Hu). Shanghai, China: BT Laboratory. Available at: https://www.bt-laboratory.com (Accessed: 26 May 2025).

Bello, A.K., Okpechi, I.G., Osman, M.A., Cho, Y., Htay, H., Jha, V., Wainstein, M. and Johnson, D.W. (2022) 'Epidemiology of haemodialysis outcomes', *Nature Reviews Nephrology*, 18(6), pp. 378–395. Available at: https://doi.org/10.1038/s41581-022-00542-7.

Bellomo, R., Kellum, J.A. and Ronco, C. (2012) 'Acute kidney injury.', *Lancet (London, England)*, 380(9843), pp. 756–766. Available at: https://doi.org/10.1016/S0140-6736(11)61454-2.

Beng, T.S., Yun, L.A., Yi, L.X., Yan, L.H., Peng, N.K., Kun, L.S., Zainuddin, S.I., Chin, L.E. and Loong, L.C. (2019) 'The experiences of suffering of end-stage renal failure patients in Malaysia: A thematic analysis', *Annals of Palliative Medicine*, 8(4), pp. 401–410. Available at:

https://doi.org/10.21037/apm.2019.03.04.

Bienaimé, F., Dechartres, A., Anglicheau, D., Sabbah, L., Montgermont, P., Friedlander, G., Ravaud, P., Legendre, C. and Prié, D. (2017) 'The Association Between Fibroblast Growth Factor 23 and Renal Transplantation Outcome Is Modified by Follow-up Duration and Glomerular Filtration Rate Assessment Method', *Kidney International Reports*, 2(5), pp. 881–892. Available at: https://doi.org/https://doi.org/10.1016/j.ekir.2017.05.007.

Bikos, A., Angeloudi, E., Memmos, E., Loutradis, C., Karpetas, A., Ginikopoulou, E., Panagoutsos, S., Pasadakis, P., Liakopoulos, V., Papagianni, A. and Sarafidis, P. (2018) 'A Comparative Study of Short-Term Blood Pressure Variability in Hemodialysis Patients with and without Intradialytic Hypertension.', *American journal of nephrology*, 48(4), pp. 295–305. Available at: https://doi.org/10.1159/000493989.

Bleicher, A. V, Unger, H.W., Rogerson, S.J. and Aitken, E.H. (2018) 'A sandwich enzyme-linked immunosorbent assay for the quantitation of human plasma ferritin.', *MethodsX*, 5, pp. 648–651. Available at: https://doi.org/10.1016/j.mex.2018.06.010.

Bondar, I.A., Klimontov, V. V. and Simakova, A.I. (2011) 'Obesity and chronic kidney disease', *Terapevticheskii Arkhiv*, 83(6), pp. 66–70.

B**onomo, J.A., Palmer, N.D., Hicks, P.J., Lea, J.P., Okusa, M.D., Langefeld, C.D., Bowden, D.W. and Freedman, B.I.** (2014) 'Complement factor H gene associations with end-stage kidney disease in African Americans', *Nephrology Dialysis Transplantation*, 29(7), pp. 1409–1414. Available at: https://doi.org/10.1093/ndt/gfu036.

Caglar, K., Peng, Y., Pupim, L.B., Flakoll, P.J., Levenhagen, D., Hakim, R.M. and Ikizler, T.A. (2002) 'Inflammatory signals associated with hemodialysis', *Kidney International*, 62(4), pp. 1408–1416. Available at: https://doi.org/https://doi.org/10.1111/j.1523-1755.2002.kid556.x.

Calvo-Lobo, C., Almazán-Polo, J., Becerro-de-Bengoa-Vallejo, R., Losa-Iglesias, M.E., Palomo-López, P., Rodríguez-Sanz, D. and López-López, D. (2019) 'Ultrasonography comparison of diaphragm thickness and excursion between athletes with and without lumbopelvic pain.', *Physical therapy in sport : official journal of the Association of Chartered Physiotherapists in Sports Medicine*, 37, pp. 128–137. Available at: https://doi.org/10.1016/j.ptsp.2019.03.015.

Calvo-Lobo, C., Neyra-Bohorquez, P.P. and Seco-Calvo, J. (2019) 'Aerobic

exercise effects in renal function and quality of life of patients with advanced chronic kidney disease', *Revista da Associacao Medica Brasileira*, 65(5), pp. 657–662. Available at: https://doi.org/10.1590/1806-9282.65.5.657.

Campo, S., Lacquaniti, A., Trombetta, D., Smeriglio, A. and Monardo, P. (2022) 'Immune System Dysfunction and Inflammation in Hemodialysis Patients: Two Sides of the Same Coin.', *Journal of clinical medicine*, 11(13). Available at: https://doi.org/10.3390/jcm11133759.

Chan, K.E., Maddux, F.W., Tolkoff-Rubin, N., Karumanchi, S.A., Thadhani, R. and Hakim, R.M. (2011) 'Early outcomes among those initiating chronic dialysis in the united states', *Clinical Journal of the American Society of Nephrology*, 6(11), pp. 2642–2649. Available at: https://doi.org/10.2215/CJN.03680411.

Chaudhary, R. (2016) 'Bacteriology of Urinary Tract Infection of Chronic Renal Failure Patients Undergoing for Hemodialysis', *Journal of Microbiology & Experimentation*, 3(3), pp. 70–74. Available at: https://doi.org/10.15406/jmen.2016.03.00089.

Chawla, L.S., Eggers, P.W., Star, R.A. and Kimmel, P.L. (2014) 'Acute kidney injury and chronic kidney disease as interconnected syndromes.', *The New England journal of medicine*, 371(1), pp. 58–66. Available at: https://doi.org/10.1056/NEJMra1214243.

Chen, C.H., Struemph, T., Jovanovich, A. and Section, R. (2021) 'FGF23 and kidney disease', in. Elsevier, pp. 115–131. Available at: https://doi.org/10.1016/B978-0-12-818036-5.00012-4.

Chen, H.-Y., Fang, W.-C., Chu, S.-C., Wang, P.-H., Lee, C.-C., Wu, I.-W., Sun, C.-Y., Hsu, H.-J., Chen, C.-Y., Chen, Y.-C., Wu, V.-C. and Pan, H.-C. (2022) 'Circulating Fibroblast Growth Factor-23 Levels Can Predict Rapid Kidney Function Decline in a Healthy Population: A Community-Based Study.', *Biomolecules*, 13(1). Available at: https://doi.org/10.3390/biom13010031.

Chen, Y.T., Jenq, C.C., Hsu, C.K., Yu, Y.C., Chang, C.H., Fan, P.C., Pan, H.C., Wu, I.W., Cherng, W.J. and Chen, Y.C. (2020) 'Acute kidney disease and acute kidney injury biomarkers in coronary care unit patients', *BMC Nephrology*, 21(1), pp. 1–11. Available at: https://doi.org/10.1186/s12882-020-01872-z.

Chew-Harris, J., Appleby, S., Richards, A.M., Troughton, R.W. and Pemberton, C.J. (2019) 'Analytical, biochemical and clearance considerations of soluble urokinase plasminogen activator receptor (suPAR) in healthy individuals',

References

Clinical Biochemistry, 69, pp. 36–44. Available at: https://doi.org/https://doi.org/10.1016/j.clinbiochem.2019.05.010.

Choi, H.J., Jeong, S.H., Shin, K.S., Kim, Y.A., Kim, Y.R., Kim, H.S., Shin, Jong **Hee, Shin, Jeong Hwan, Uh, Y., Bae, S., Yoon, E.-J. and Yoo, J.S.** (2022) 'Characteristics of Escherichia coli Urine Isolates and Risk Factors for Secondary Bloodstream Infections in Patients with Urinary Tract Infections.', *Microbiology spectrum*, 10(4), p. e0166022. Available at: https://doi.org/10.1128/spectrum.01660-22.

Coemans, M., Süsal, C., Döhler, B., Anglicheau, D., Giral, M., Bestard, O., Legendre, C., Emonds, M.-P., Kuypers, D., Molenberghs, G., Verbeke, G. and Naesens, M. (2018) 'Analyses of the short- and long-term graft survival after kidney transplantation in Europe between 1986 and 2015.', *Kidney international*, 94(5), pp. 964–973. Available at: https://doi.org/10.1016/j.kint.2018.05.018.

Cristea, O.M., Avrămescu, C.S., Bălășoiu, M., Popescu, F.D., Popescu, F. and Amzoiu, M.O. (2017) 'Urinary tract infection with Klebsiella pneumoniae in Patients with Chronic Kidney Disease.', *Current health sciences journal*. Romania, pp. 137–148. Available at: https://doi.org/10.12865/CHSJ.43.02.06.

Czaya, B. and Faul, C. (2019) 'The Role of Fibroblast Growth Factor 23 in Inflammation and Anemia.', *International journal of molecular sciences*, 20(17). Available at: https://doi.org/10.3390/ijms20174195.

Davison, S.N. (2005) 'Chronic pain in end-stage renal disease', *Advances in Chronic Kidney Disease*, 12(3), pp. 326–334. Available at: https://doi.org/10.1016/j.ackd.2005.03.008.

Desmedt, S., Desmedt, V., Delanghe, J.R., Speeckaert, R. and Speeckaert, M.M. (2017) 'The intriguing role of soluble urokinase receptor in inflammatory diseases', *Critical Reviews in Clinical Laboratory Sciences*, 54(2), pp. 117–133. Available at: https://doi.org/10.1080/10408363.2016.1269310.

Dhanashri Santosh Mane and Pramod Motiram Bhosale (2023) 'A review on prevalence of bacteria in urinary tract infection', *World Journal of Advanced Engineering Technology and Sciences*, 14(1), pp. 231–238. Available at: https://doi.org/10.30574/wjbphs.2023.14.1.0177.

Diallo, A.Y., Diallo, Mamadou Mouctar, Barry, M.D., Barry, K.M.B., Diallo, S.O., Diallo, D., Bangoura, S., Diallo, Mamadou Malal, Diallo, T.M.O., Kaba, M.L. and Bah, A.O. (2024) 'Causes and Prognosis of Cases of Acute Obstructive Renal Failure Managed at the Donka National Hemodialysis Center', *Open Journal*

of Nephrology, 14(02), pp. 136–146. Available at: https://doi.org/10.4236/ojneph.2024.142014.

Dimitrijevic, Z., Paunovic, G., Tasic, D., Mitic, B. and Basic, D. (2021) 'Risk factors for urosepsis in chronic kidney disease patients with urinary tract infections', *Scientific Reports*, 11(1), pp. 1–8. Available at: https://doi.org/10.1038/s41598-021-93912-3.

Diniz, H. and Frazão, J.M. (2013) 'The role of fibroblast growth factor 23 in chronic kidney disease-mineral and bone disorder', *Nefrología : publicación oficial de la Sociedad Española Nefrologia*, 33(6), pp. 835–844. Available at: https://doi.org/10.3265/Nefrologia.pre2013.Jul.12091.

Donadei, C., Angeletti, A., Pizzuti, V., Zappulo, F., Conte, D., Cappuccilli, M., Chiocchini, A.L., Scrivo, A., Apuzzo, D., Mariggiò, M.A., Gasperoni, L., Donati, G. and La Manna, G. (2023) 'Impact of Single Hemodialysis Treatment on immune Cell Subpopulations', *Journal of Clinical Medicine*, 12(9). Available at: https://doi.org/10.3390/jcm12093107.

Drechsler, C., Hayek, S., Wei, C., Sever, S., Genser, B., Krane, V., Meinitzer, A., März, W., Wanner, C. and Reiser, J. (2017) 'Soluble Urokinase Plasminogen Activator Receptor and Outcomes in Patients with Diabetes on Hemodialysis', *Clinical Journal of the American Society of Nephrology*, 12, p. CJN.10881016. Available at: https://doi.org/10.2215/CJN.10881016.

Ducloux, D., Legendre, M., Bamoulid, J., Rebibou, J.M., Saas, P., Courivaud, C. and Crepin, T. (2018) 'ESRD-associated immune phenotype depends on dialysis modality and iron status: Clinical implications', *Immunity and Ageing*, 15(1), pp. 1–10. Available at: https://doi.org/10.1186/s12979-018-0121-z.

Eboh, C. and Chowdhury, T.A. (2015) 'Management of diabetic renal disease', *Annals of Translational Medicine*, 3(11), pp. 1–8. Available at: https://doi.org/10.3978/j.issn.2305-5839.2015.06.25.

Erbak Yılmaz, H., Aksun, S., Günay, S., Elmalı, F. and Çekiç, C. (2023) 'Evaluation of plasma soluble urokinase plasminogen activator receptor (SuPAR) levels in ulcerative colitis', *Arab Journal of Gastroenterology*, 24(3), pp. 175–179. Available at: https://doi.org/https://doi.org/10.1016/j.ajg.2023.03.001.

Esposito, P., Cappadona, F., Prenna, S., Marengo, M., Fiorentino, M., Fabbrini, P., Quercia, A.D., Naso, E., Garzotto, F., Viazzi, F., Castellano, G. and Cantaluppi, V. (2024) '#2146 Gender difference in hospital-acute kidney injury epidemiology and outcome: not just a matter of sex', *Nephrology Dialysis*

References

Transplantation, 39(Supplement_1), pp. gfae069-1121–2146. Available at: https://doi.org/10.1093/ndt/gfae069.1121.

Estakhri, R., Niaki, N.M., Noshad, H., Asghari, M. and Barghi, H. (2020) 'Diagnostic value of serum procalcitonin for diagnosis of bacterial infection in patients with chronic kidney disease under hemodialysis', *Immunopathologia Persa*, 6(1), pp. 1–6. Available at: https://doi.org/10.15171/ipp.2020.08.

Evans, M., Lewis, R.D., Morgan, A.R., Whyte, M.B., Hanif, W., Bain, S.C., Davies, S., Dashora, U., Yousef, Z., Patel, D.C. and Strain, W.D. (2022) 'A Narrative Review of Chronic Kidney Disease in Clinical Practice: Current Challenges and Future Perspectives', *Advances in Therapy*, 39(1), pp. 33–43. Available at: https://doi.org/10.1007/s12325-021-01927-z.

Fayed, A., Radwan, W.A., Amin, M. and Gamal, A. (2019) 'Prediction of Mortality and Need for Renal Replacement Therapy in Patients of Acute Kidney Injury Using Fibroblast Growth Factor 23', *Saudi Journal of Kidney Diseases and Transplantation*, 30(5). Available at: https://journals.lww.com/sjkd/fulltext/2019/30050/prediction_of_mortality_and_need_for_renal.6.aspx.

Fearn, A. and Sheerin, N.S. (2015) 'Complement activation in progressive renal disease.', *World journal of nephrology*, 4(1), pp. 31–40. Available at: https://doi.org/10.5527/wjn.v4.i1.31.

Fenner, A. (2015) 'Dysregulation of E. coli α-hemolysin alters UTI course', *Nature Reviews Urology*, 12(4), p. 179. Available at: https://doi.org/10.1038/nrurol.2015.32.

Fernández-Espigares, L., Hernández-Chico, I., Expósito-Ruiz, M., Rosales-Castillo, A., Navarro-Marí, J.M. and Gutiérrez-Fernández, J. (2023) 'Antibiotic Resistance Changes in Gram-Positive Bacteria from Urine Cultures: Development Analysis in a Health Area of South-East Spain', *Antibiotics*, 12(7). Available at: https://doi.org/10.3390/antibiotics12071133.

Fiseha, T. and Osborne, N.J. (2023) 'Burden of end-stage renal disease of undetermined etiology in Africa', *Renal Replacement Therapy*, 9(1), pp. 1–10. Available at: https://doi.org/10.1186/s41100-023-00497-w.

Foresto-Neto, O., Menezes-Silva, L., Leite, J.A., Andrade-Silva, M. and Câmara, N.O.S. (2024) 'Immunology of Kidney Disease', *Annual review of immunology*, 42(1), pp. 207–233. Available at: https://doi.org/10.1146/annurev-immunol-090122-045843.

References

Forbes, BA, Sahm, DF & Weissfeld, AS (2014) Bailey & Scott's diagnostic microbiology. 13th edn. St. Louis, Missouri: Elsevier Mosby.

Francis, A., Harhay, M.N., Ong, A.C.M., Tummalapalli, S.L., Ortiz, A., Fogo, A.B., Fliser, D., Roy-Chaudhury, P., Fontana, M., Nangaku, M., Wanner, C., Malik, C., Hradsky, A., Adu, D., Bavanandan, S., Cusumano, A., Sola, L., Ulasi, I. and Jha, V. (2024) 'Chronic kidney disease and the global public health agenda: an international consensus', *Nature Reviews Nephrology*, 20(7), pp. 473–485. Available at: https://doi.org/10.1038/s41581-024-00820-6.

Franzin, R., Stasi, A., Caggiano, G., Squiccimarro, E., Losappio, V., Fiorentino, M., Alfieri, C., Stallone, G., Gesualdo, L. and Castellano, G. (2023) 'Enhancing Immune Protection in Hemodialysis Patients: Role of the Polymethyl Methacrylate Membrane', *Blood Purification*, 52(suppl 1), pp. 49–61. Available at: https://doi.org/10.1159/000529971.

Fysaraki, M., Samonis, G., Valachis, A., Daphnis, E., Karageorgopoulos, D.E., Falagas, M.E., Stylianou, K. and Kofteridis, D.P. (2013) 'Incidence, clinical, microbiological features and outcome of bloodstream infections in patients undergoing hemodialysis.', *International journal of medical sciences*, 10(12), pp. 1632–1638. Available at: https://doi.org/10.7150/ijms.6710.

Gajdács, M., Ábrók, M., Lázár, A. and Burián, K. (2020) 'Increasing relevance of Gram-positive cocci in urinary tract infections: a 10-year analysis of their prevalence and resistance trends', *Scientific Reports*, 10(1), pp. 1–11. Available at: https://doi.org/10.1038/s41598-020-74834-y.

Ganz, T., Aronoff, G.R., Gaillard, C.A.J.M., Goodnough, L.T., Macdougall, I.C., Mayer, G., Porto, G., Winkelmayer, W.C. and Wish, J.B. (2020) 'Iron Administration, Infection, and Anemia Management in CKD: Untangling the Effects of Intravenous Iron Therapy on Immunity and Infection Risk.', *Kidney medicine*, 2(3), pp. 341–353. Available at: https://doi.org/10.1016/j.xkme.2020.01.006.

García, Á., Aggarwal, C.C. and Tang, Z.C. (2023) 'ADAMTS7-Mediated Complement Factor H Degradation Potentiates Complement Activation to Contributing to Renal Injuries', *Journal of the American Society of Nephrology*, 34(2), pp. 291–308. Available at: https://doi.org/10.1681/asn.0000000000000004.

Gattia, K.J., Mohammed, H.Q. and Ahmed, T.H. (2014) 'Study of some physiological parameters in renal failure patients', *Wasit Journal for Science & Medicine*, 7(2), pp. 1–12.

References

Gebretensaie, Y., Atnafu, A., Girma, S., Alemu, Y. and Desta, K. (2023) 'Prevalence of Bacterial Urinary Tract Infection, Associated Risk Factors, and Antimicrobial Resistance Pattern in Addis Ababa, Ethiopia: A Cross-Sectional Study', *Infection and Drug Resistance*, 16(May), pp. 3041–3050. Available at: https://doi.org/10.2147/IDR.S402279.

Georgianos, P.I. and Agarwal, R. (2023) 'Hypertension in chronic kidney disease—treatment standard 2023', *Nephrology Dialysis Transplantation*, 38(12), pp. 2694–2703. Available at: https://doi.org/10.1093/ndt/gfad118.

Go, A.S., Hsu, C.-Y., Yang, J., Tan, T.C., Zheng, S., Ordonez, J.D. and Liu, K.D. (2018) 'Acute Kidney Injury and Risk of Heart Failure and Atherosclerotic Events.', *Clinical journal of the American Society of Nephrology : CJASN*, 13(6), pp. 833–841. Available at: https://doi.org/10.2215/CJN.12591117.

Goetz, R., Beenken, A., Ibrahimi, O.A., Kalinina, J., Olsen, S.K., Eliseenkova, A. V, Xu, C., Neubert, T.A., Zhang, F., Linhardt, R.J., Yu, X., White, K.E., Inagaki, T., Kliewer, S.A., Yamamoto, M., Kurosu, H., Ogawa, Y., Kuro-o, M., Lanske, B., Razzaque, M.S. and Mohammadi, M. (2007) 'Molecular Insights into the Klotho-Dependent, Endocrine Mode of Action of Fibroblast Growth Factor 19 Subfamily Members', *Molecular and Cellular Biology*, 27(9), pp. 3417–3428. Available at: https://doi.org/10.1128/MCB.02249-06.

Gómez Delgado, I. and Sánchez-Corral, P. (2022) 'Contribution of functional and quantitative genetic variants of Complement Factor H and Factor H-Related (FHR) proteins on renal pathology', *Nefrologia*, 42(3), pp. 280–289. Available at: https://doi.org/10.1016/j.nefro.2021.07.003.

González-Cuadrado, C., Caro-Espada, P.J., Chivite-Lacaba, M., Utrero-Rico, A., Lozano-Yuste, C., Gutierrez-Solis, E., Morales, E., Sandino-Pérez, J., Gil-Etayo, F.J., Allende-Martínez, L., Laguna-Goya, R. and Paz-Artal, E. (2023) 'Hemodialysis-Associated Immune Dysregulation in SARS-CoV-2-Infected End-Stage Renal Disease Patients', *International Journal of Molecular Sciences*, 24(2). Available at: https://doi.org/10.3390/ijms24021712.

Griveas, I., Schoinas, A., Balitsari, A., Asimakopoulos, G. and Pratilas, E. (2021) 'Soluble urokinase plasminogen activator receptor and its complicated role in hemodialysis (HD) patients with Covid-19 infection', *Asian Journal of Medical Sciences*, 12(7), pp. 1–4. Available at: https://doi.org/10.3126/ajms.v12i7.36499.

Gutiérrez, O.M., Mannstadt, M., Isakova, T., Rauh-Hain, J.A., Tamez, H., Shah, A., Smith, K., Lee, H., Thadhani, R., Jüppner, H. and Wolf, M. (2008) 'Fibroblast growth factor 23 and mortality among patients undergoing

References

hemodialysis.', *The New England journal of medicine*, 359(6), pp. 584–592. Available at: https://doi.org/10.1056/NEJMoa0706130.

Habas, E., Habas, A., Elgamal, M., Shraim, B., Moursi, M., Ibrahim, A., Danjuma, M. and Elzouki, A.-N. (2021) 'Common complications of hemodialysis: A clinical review', *Ibnosina Journal of Medicine and Biomedical Sciences*, 13(04), pp. 161–172. Available at: https://doi.org/10.4103/ijmbs.ijmbs_62_21.

Haider, J.S. (2016) 'Frequency of Urinary Tract Bacterial Infection and their Susceptibility Patterns among Hemodialysis Patients in Zliten Hospital', *Journal of Microbiology & Experimentation*, 3(3), pp. 93–97. Available at: https://doi.org/10.15406/jmen.2016.03.00093.

Hall, J. and Linton, K.D. (2008) 'Obstruction of the upper and lower urinary tract', *Surgery - Oxford International Edition*, 26(5), pp. 197–202. Available at: https://doi.org/10.1016/j.mpsur.2008.03.007.

Hassooni, J.J., Kareem, J.J., Hassan, M.M. and Rashid, A.N. (2018) 'Detection of Most Pathogenic Bacteria in Renal Failure and Urinary Tract Infections Patients with Antibiotics Patterns', *International Journal of Biomedical Engineering*, 4(1), p. 15. Available at: https://doi.org/10.11648/J.IJBECS.20180401.13.

Hayek, S.S., Ko, Y.-A., Awad, M., Ahmed, H., Gray, B., Hosny, K.M., Aida, H., Tracy, M.J., Wei, C., Sever, S., Reiser, J. and Quyyumi, A.A. (2017) 'Cardiovascular Disease Biomarkers and suPAR in Predicting Decline in Renal Function: A Prospective Cohort Study', *Kidney International Reports*, 2(3), pp. 425–432. Available at: https://doi.org/https://doi.org/10.1016/j.ekir.2017.02.001.

Hebert, S.A. and Ibrahim, H.N. (2022) 'Hypertension Management in Patients with Chronic Kidney Disease.', *Methodist DeBakey cardiovascular journal*, 18(4), pp. 41–49. Available at: https://doi.org/10.14797/mdcvj.1119.

Hedayati, M., Abubaker-Sharif, B., Khattab, M., Razavi, A., Mohammed, I., Nejad, A., Wabler, M., Zhou, H., Mihalic, J., Gruettner, C., DeWeese, T. and Ivkov, R. (2018) 'An optimised spectrophotometric assay for convenient and accurate quantitation of intracellular iron from iron oxide nanoparticles.', *International journal of hyperthermia : the official journal of European Society for Hyperthermic Oncology, North American Hyperthermia Group*, 34(4), pp. 373–381. Available at: https://doi.org/10.1080/02656736.2017.1354403.

Himmelfarb, J., Vanholder, R., Mehrotra, R. and Tonelli, M. (2020) 'The current and future landscape of dialysis.', *Nature reviews. Nephrology*, 16(10), pp.

573–585. Available at: https://doi.org/10.1038/s41581-020-0315-4.

Huang, Y., Huang, S., Zhuo, X. and Lin, M. (2023) 'Predictive value of suPAR in AKI: a systematic review and meta-analysis.', *Clinical and experimental nephrology*, 27(1), pp. 1–11. Available at: https://doi.org/10.1007/s10157-022-02300-2.

Hussain, I., Tandi, R., Singh, G., Kaur, G., Abhishek, Dodda, S., Patel, D., Natarajan, B., Maram, T., Kedia, A., Vempati, R., Sahu, S. and Choubey, U. (2023) 'Correlation of FGF-23 With Biochemical Markers and Bone Density in Chronic Kidney Disease-Bone Mineral Density Disorder.', *Cureus*, 15(1), p. e33879. Available at: https://doi.org/10.7759/cureus.33879.

Hwang, J.H., Park, H.C., Jeong, J.C., Ha Baek, S., Han, M.Y., Bang, K., Cho, J.Y., Yu, S.H., Yang, J., Oh, K.H., Hwang, Y.H. and Ahn, C. (2013) 'Chronic asymptomatic pyuria precedes overt urinary tract infection and deterioration of renal function in autosomal dominant polycystic kidney disease', *BMC Nephrology*, 14(1), pp. 1–9. Available at: https://doi.org/10.1186/1471-2369-14-1.

Iamartino, L. and Brandi, M.L. (2022) 'The calcium-sensing receptor in inflammation: Recent updates', *Frontiers in Physiology*, 13. Available at: https://doi.org/10.3389/fphys.2022.1059369.

Ibrahim, S. and Rashed, L.A. (2009) 'Serum fibroblast growth factor-23 levels in chronic haemodialysis patients.', *International Urology and Nephrology*, 41(1), pp. 163–169. Available at: https://doi.org/10.1007/S11255-008-9466-0.

Inagi, R., Kumagai, T., Nishi, H., Kawakami, T., Miyata, T., Fujita, T. and Nangaku, M. (2008) 'Preconditioning with Endoplasmic Reticulum Stress Ameliorates Mesangioproliferative Glomerulonephritis', *Journal of the American Society of Nephrology*, 19(5). Available at: https://journals.lww.com/jasn/fulltext/2008/05000/preconditioning_with_endoplasmic_reticulum_stress.15.aspx.

Iqbaal, K., Aslam, R., Iqbal, S., Munir, N., Abdullah, I., Rasheed, S., Ajmal, A., Ali, M. and Ali, A. (2024) 'Analyzing the Impact of Hemodialysis on Urea and Creatinine analytes in Renal Failure Patients from Lahore-Pakistan', 5, pp. 27–32. Available at: https://doi.org/10.52587/njmhs.v5i1.65.

Isakova, T., Xie, H., Yang, W., Xie, D., Anderson, A.H., Scialla, J., Wahl, P., Gutiérrez, O.M., Steigerwalt, S., He, J., Schwartz, S., Lo, J., Ojo, A., Sondheimer, J., Hsu, C., Lash, J., Leonard, M., Kusek, J.W., Feldman, H.I., Wolf, M. and Chronic Renal Insufficiency Cohort (CRIC) Study Group, for the

References

(2011) ‘Fibroblast Growth Factor 23 and Risks of Mortality and End-Stage Renal Disease in Patients With Chronic Kidney Disease’, *JAMA*, 305(23), pp. 2432–2439. Available at: https://doi.org/10.1001/jama.2011.826.

İşsever, K. and Dheir, H. (2023) ‘The Relationship Between Soluble Urokinase-Type Plasminogen Activator Receptor (suPAR) Levels and Treatment Response in Patients With Glomerulonephritis: A Single-Center Experience’, *Cureus* [Preprint]. Available at: https://doi.org/10.7759/cureus.47473.

Itoh, N., Ohta, H. and Konishi, M. (2015) ‘Endocrine FGFs: Evolution, Physiology, Pathophysiology, and Pharmacotherapy.’, *Frontiers in endocrinology*, 6, p. 154. Available at: https://doi.org/10.3389/fendo.2015.00154.

Iversen, E., Houlind, M.B., Kallemose, T., Rasmussen, L.J.H., Hornum, M., Feldt-Rasmussen, B., Hayek, S.S., Andersen, O. and Eugen-Olsen, J. (2020) ‘Elevated suPAR Is an Independent Risk Marker for Incident Kidney Disease in Acute Medical Patients’, *Frontiers in Cell and Developmental Biology*, 8(June), pp. 1–9. Available at: https://doi.org/10.3389/fcell.2020.00339.

Jehn, U., Schütte-Nütgen, K., Henke, U., Pavenstädt, H., Suwelack, B. and Reuter, S. (2021) ‘Soluble urokinase-type plasminogen activator receptor (suPAR) is a risk indicator for eGFR loss in kidney transplant recipients’, *Scientific Reports*, 11(1), pp. 1–9. Available at: https://doi.org/10.1038/s41598-021-83333-7.

Jha, R., Lopez-Trevino, S., Kankanamalage, H.R. and Jha, J.C. (2024) ‘Diabetes and Renal Complications: An Overview on Pathophysiology, Biomarkers and Therapeutic Interventions’, *Biomedicines*, 12(5). Available at: https://doi.org/10.3390/biomedicines12051098.

Karkar, A. (2018) ‘Infection control guidelines in hemodialysis facilities’, *Kidney Res Clin Pract*, 37(1), pp. 1–3. Available at: https://doi.org/10.23876/j.krcp.2018.37.1.1.

Kato, G.J., Steinberg, M.H. and Gladwin, M.T. (2017) ‘Intravascular hemolysis and the pathophysiology of sickle cell disease.’, *The Journal of clinical investigation*, 127(3), pp. 750–760. Available at: https://doi.org/10.1172/JCI89741.

Kawabata, C., Komaba, H., Ishida, H., Nakagawa, Y., Hamano, N., Koizumi, M., Kanai, G., Wada, T., Nakamura, M. and Fukagawa, M. (2020) ‘Changes in Fibroblast Growth Factor 23 and Soluble Klotho Levels After Hemodialysis Initiation’, *Kidney Medicine*, 2(1), pp. 59–67. Available at: https://doi.org/https://doi.org/10.1016/j.xkme.2019.09.007.

Kesarwani, V., Bukhari, M.H., Kahlenberg, J.M. and Wang, S. (2024) ‘Urinary

complement biomarkers in immune-mediated kidney diseases', (June), pp. 1–15. Available at: https://doi.org/10.3389/fimmu.2024.1357869.

Kiran, B., Prema, A., Thilagavathi, R. and Jamuna Rani, R. (2014) 'Serum 25-Hydroxy Vitamin D, calcium, phosphorus and alkaline phosphatase levels in healthy adults above the age of 20 living in Potheri Village of Kancheepuram District , Tamilnadu', *Journal of Applied Pharmaceutical Science*, 4(12), pp. 030–034. Available at: https://doi.org/10.7324/JAPS.2014.41206.

Ku, E., Lee, B.J., Wei, J. and Weir, M.R. (2019) 'Hypertension in CKD: Core Curriculum 2019', *American Journal of Kidney Diseases*, 74(1), pp. 120–131. Available at: https://doi.org/10.1053/j.ajkd.2018.12.044.

Kuczera, P., Maszczyk, A., Machura, E., Kurzak, E., Adamczak, M. and Więcek, A. (2015) 'Serum parathyroid hormone concentrations measured by chemiluminescence and electrochemiluminescence methods — are the results comparable in haemodialysis patients with chronic kidney disease?', *Endokrynologia Polska*, 66(3), pp. 219–223. Available at: https://doi.org/10.5603/EP.2015.0028.

Lamarche, C., Iliuta, I.-A. and Kitzler, T. (2019) 'Infectious Disease Risk in Dialysis Patients: A Transdisciplinary Approach', *Canadian Journal of Kidney Health and Disease*, 6, p. 2054358119839080. Available at: https://doi.org/10.1177/2054358119839080.

Latic, N. and Erben, R.G. (2021) 'FGF23 and Vitamin D Metabolism', *JBMR Plus*, 5(12), pp. 1–7. Available at: https://doi.org/10.1002/jbm4.10558.

Laville, S.M., Couturier, A., Lambert, O., Metzger, M., Mansencal, N., Jacquelinet, C., Laville, M., Frimat, L., Fouque, D., Combe, C., Robinson, B.G., Stengel, B., Liabeuf, S. and Massy, Z.A. (2022) 'Urea levels and cardiovascular disease in patients with chronic kidney disease', *Nephrology Dialysis Transplantation*, 38(1), pp. 184–192. Available at: https://doi.org/10.1093/ndt/gfac045.

Leone, S. and Suter, F. (2010) 'Severe bacterial infections in haemodialysis patients', *Infezioni in Medicina*, 18(2), pp. 79–85.

Levey, A.S., Levin, A. and Kellum, J.A. (2013) 'Definition and Classification of Kidney Diseases', *American Journal of Kidney Diseases*, 61(5), pp. 686–688. Available at: https://doi.org/10.1053/j.ajkd.2013.03.003.

Lin, Y.-C., Chang, Y.-H., Yang, S.-Y., Wu, K.-D. and Chu, T.-S. (2018) 'Update of pathophysiology and management of diabetic kidney disease', *Journal of the*

References

Formosan Medical Association, 117(8), pp. 662–675. Available at: https://doi.org/https://doi.org/10.1016/j.jfma.2018.02.007.

Liu, C., Debnath, N., Mosoyan, G., Chauhan, K., Vasquez-Rios, G., Soudant, C., Menez, S., Parikh, C.R. and Coca, S.G. (2022) 'Systematic Review and Meta-Analysis of Plasma and Urine Biomarkers for CKD Outcomes.', *Journal of the American Society of Nephrology : JASN*, 33(9), pp. 1657–1672. Available at: https://doi.org/10.1681/ASN.2022010098.

Losappio, V., Franzin, R., Infante, B., Godeas, G., Gesualdo, L., Fersini, A., Castellano, G. and Stallone, G. (2020) 'Molecular mechanisms of premature aging in hemodialysis: The complex interplay between innate and adaptive immune dysfunction', *International Journal of Molecular Sciences*, 21(10), pp. 1–24. Available at: https://doi.org/10.3390/ijms21103422.

Ma, X., Zhang, F., Bai, B., Lin, Z., Xu, G., Chen, Z., Sun, X., Zheng, J., Deng, Q. and Yu, Z. (2021) 'Linezolid Resistance in Enterococcus faecalis Associated With Urinary Tract Infections of Patients in a Tertiary Hospitals in China: Resistance Mechanisms, Virulence, and Risk Factors', *Frontiers in Public Health*, 9(February). Available at: https://doi.org/10.3389/fpubh.2021.570650.

Mahajan, S., Jacob, A., Kelkar, A., Chang, A., Mcskimming, D., Neelamegham, S., Quigg, R.J. and Alexander, J.J. (2021) 'Local complement factor H protects kidney endothelial cell structure and function', *Kidney International*, 100(4), pp. 824–836. Available at: https://doi.org/https://doi.org/10.1016/j.kint.2021.05.033.

Mahmood, M.M. (2023) 'Imbalances in parathyroid hormones and few electrolytes in patients with renal failure', *Biomedicine* [Preprint]. Available at: https://doi.org/10.51248/.v43i3.2820.

Maina, D. and Kagotho, E. (2014) 'SUITABILITY OF VITEK 2 SYSTEM IN IDENTIFICATION AND SUSCEPTIBILITY TESTING OF GRAM NEGATIVE BACTEREMIAS BY DIRECT INOCULATION.', *East African medical journal*, 91(4), pp. 115–118.

Majeed, H.T. and Aljanaby, A.A.J. (2019) 'Antibiotic Susceptibility Patterns and Prevalence of Some Extended Spectrum Beta-Lactamases Genes in Gram-Negative Bacteria Isolated from Patients Infected with Urinary Tract Infections in Al-Najaf City, Iraq.', *Avicenna journal of medical biotechnology*, 11(2), pp. 192–201.

Makris, K. and Spanou, L. (2016) 'Acute Kidney Injury: Definition, Pathophysiology and Clinical Phenotypes.', *The Clinical biochemist. Reviews*,

37(2), pp. 85–98.

Malkina, A. (2023) 'Overview of Kidney Failure'. MSD Manual. Available at: https://www.msdmanuals.com/home/kidney-and-urinary-tract-disorders/kidney-failure/overview-of-kidney-failure.

Madigan, MT, Bender, KS, Buckley, DH, Sattley, WM & Stahl, DA (2019) Brock biology of microorganisms. 15th edn. New York: Pearson.

Manhal, F.S., Mohammed, A.A. and Ali, K.H. (2012) 'Urinary tract infection in Hemodialysis patients with renal failure', *Journal of the Faculty of Medicine Baghdad*, 54(1), pp. 38–41. Available at: https://doi.org/10.32007/jfacmedbagdad.541768.

Matsui, I., Oka, T., Kusunoki, Y., Mori, D., Hashimoto, N., Matsumoto, A., Shimada, K., Yamaguchi, S., Kubota, K., Yonemoto, S., Higo, T., Sakaguchi, Y., Takabatake, Y., Hamano, T. and Isaka, Y. (2018) 'Cardiac hypertrophy elevates serum levels of fibroblast growth factor 23', *Kidney International*, 94(1), pp. 60–71. Available at: https://doi.org/10.1016/j.kint.2018.02.018.

Mehmood, Y., Ali, I., Zahra, K. and Ashraf, U. (2019) 'HEMODIALYSIS';, *The Professional Medical Journal*, 26. Available at: https://doi.org/10.29309/TPMJ/2019.26.01.2511.

Mei Tao Danna Zheng, X.L.Q.H. and Zhang, W. (2022) 'Diagnostic value of procalcitonin for bacterial infections in patients undergoing hemodialysis: a systematic review and meta-analysis', *Renal Failure*, 44(1), pp. 81–93. Available at: https://doi.org/10.1080/0886022X.2021.2021236.

Mendoza, J., Isakova, T., Ricardo, A.C., Xie, H., Navaneethan, S.D., Anderson, A.H., Bazzano, L.A., Xie, D., Kretzler, M., Nessel, L., Hamm, L.L., Negrea, L., Leonard, M.B., Raj, D.S. and Wolf, M. (2012) 'Fibroblast growth factor 23 and Inflammation in CKD', *Clinical Journal of The American Society of Nephrology*, 7(7), pp. 1155–1162. Available at: https://doi.org/10.2215/CJN.13281211.

Micarelli, D., Cristi, E., Taddei, A.R., Rovere, F.R. Della, Mercanti, C. and Feriozzi, S. (2020) 'A case of acute renal failure with multiple origins of the renal injury.', *CEN case reports*, 9(4), pp. 437–441. Available at: https://doi.org/10.1007/s13730-020-00505-6.

Mohammed, G.J., Kadhim, M.J. and Hameed, I.H. (2016) 'Proteus species: Characterization and herbal antibacterial: A review', *International Journal of Pharmacognosy and Phytochemical Research*, 8(11), pp. 1844–1854.

References

Mohsen, I., Maaroof, R. and Alduhaidhawi, A. (2023) 'Renal Failure, Types, Causes and Etiology: A Review Article', *INTERNATIONAL JOURNAL OF MEDICAL SCIENCE AND CLINICAL RESEARCH STUDIES*, 03. Available at: https://doi.org/10.47191/ijmscrs/v3-i8-41.

Mollahosseini, A., Abdelrasoul, A. and Shoker, A. (2020) 'A critical review of recent advances in hemodialysis membranes hemocompatibility and guidelines for future development', *Materials Chemistry and Physics*, 248, p. 122911. Available at: https://doi.org/https://doi.org/10.1016/j.matchemphys.2020.122911.

Mousa, Y., Kheder, M., Moenes, H. and Shehata, M. (2022) 'The relation between fibroblast growth factor 23 level and anemia in chronic kidney disease patients.', *Minia Journal of Medical Research*, 0(0), pp. 33–38. Available at: https://doi.org/10.21608/mjmr.2022.217059.

Munoz Mendoza, J., Isakova, T., Ricardo, A.C., Xie, H., Navaneethan, S.D., Anderson, A.H., Bazzano, L.A., Xie, D., Kretzler, M., Nessel, L., Hamm, L.L., Negrea, L., Leonard, M.B., Raj, D., Wolf, M. and Cohort, for the C.R.I. (2012) 'Fibroblast Growth Factor 23 and Inflammation in CKD', *Clinical Journal of the American Society of Nephrology*, 7(7). Available at: https://journals.lww.com/cjasn/fulltext/2012/07000/fibroblast_growth_factor_23_and_inflammation_in.15.aspx.

Jones, A. & Patel, R. (2020) 'Growth of streptococci on blood agar', Journal of Clinical Microbiology, 58(3), pp. 150-155. Figure 2, p. 152.

Murdeshwar, H.N. and Anjum, F. (2024) 'Hemodialysis.', in. Treasure Island (FL).

N., S.J. and M., P.M. (2015) 'Proteus mirabilis and Urinary Tract Infections', *Microbiology Spectrum*, 3(5), pp. 10.1128/microbiolspec.uti-0017–2013. Available at: https://doi.org/10.1128/microbiolspec.uti-0017-2013.

Nagamachi, S., Ohsawa, I., Suzuki, H., Sato, N., Inoshita, H., Hisada, A., Honda, D., Shimamoto, M., Shimizu, Y., Horikoshi, S. and Tomino, Y. (2014) 'Properdin has an ascendancy over factor H regulation in complement-mediated renal tubular damage', *BMC Nephrology*, 15(1), p. 82. Available at: https://doi.org/10.1186/1471-2369-15-82.

Naskar, M. and Choi, H.W. (2024) 'A Dynamic Interplay of Innate Immune Responses During Urinary Tract Infection', *Immune Network*, 24(4). Available at: https://doi.org/10.4110/in.2024.24.e31.

Nathan, S., Virginia, L.C., S., B.A., C., D., S., G.M. and E., J.D. (2001) 'Role of

References

Enterococcus faecalisSurface Protein Esp in the Pathogenesis of Ascending Urinary Tract Infection', *Infection and Immunity*, 69(7), pp. 4366–4372. Available at: https://doi.org/10.1128/iai.69.7.4366-4372.2001.

Nguyen, D.B., Arduino, M.J. and Patel, P.R. (2019) 'Hemodialysis-Associated Infections.', *Chronic Kidney Disease, Dialysis, and Transplantation*, pp. 389-410.e8. Available at: https://doi.org/10.1016/B978-0-323-52978-5.00025-2.

Ni, W., Han, Y., Zhao, J., Cui, J., Wang, K., Wang, R. and Liu, Y. (2016) 'Serum soluble urokinase-Type plasminogen activator receptor as a biological marker of bacterial infection in adults: A systematic review and meta-Analysis', *Scientific Reports*, 6(December), pp. 1–8. Available at: https://doi.org/10.1038/srep39481.

Niu, X., Hou, B., Yang, L., Wang, W., Yu, Q., Mao, M. and Shen, W. (2023) 'Patterns of Drug Resistance and Bacterial Pathogen Distribution in Patients with Urinary Tract Infections in the Jiaxing Region from 2020 to 2022', (August), pp. 5911–5921.

Obeagu, E.I., Eze, V.U., Alaeboh, E.A. and Ochei, K.C. (2016) 'Determination of haematocrit level and iron profile study among persons living with HIV in Umuahia, Abia State, Nigeria', *J BioInnovation*, 5(4), pp. 464–471.

Onyebueke, E.A., Onyemelukwe, N.F. and Oladeji, D.S. (2019) 'Antibiotic susceptibility pattern of staphylococcus species implicated in urinary tract infection in enugu state nigeria', *Pharmacologyonline*, 1, pp. 166–176.

Pawlak, K., Pawlak, D. and Mysliwiec, M. (2007) 'Excess soluble urokinase-type plasminogen activator receptor in the plasma of dialysis patients correlates with increased fibrinolytic activity', *Thrombosis Research*, 119(4), pp. 475–480. Available at: https://doi.org/https://doi.org/10.1016/j.thromres.2006.03.011.

Peitzman, S.J. (2001) 'Chronic dialysis and dialysis doctors in the United States: A nephrologist-historian's perspective', *Seminars in Dialysis*, 14(3), pp. 200–208. Available at: https://doi.org/10.1046/j.1525-139X.2001.00053.x.

Perkins, S.J., Nan, R., Okemefuna, A.I., Li, K., Khan, S. and Miller, A. (2010) 'Multiple Interactions of Complement Factor H with Its Ligands in Solution: A Progress Report', in J.D. Lambris and A.P. Adamis (eds) *Inflammation and Retinal Disease: Complement Biology and Pathology*. New York, NY: Springer New York, pp. 25–47.

Pickering, M.C. and Cook, H.T. (2008) 'Translational mini-review series on complement factor H: renal diseases associated with complement factor H: novel

insights from humans and animals.', *Clinical and experimental immunology*, 151(2), pp. 210–230. Available at: https://doi.org/10.1111/j.1365-2249.2007.03574.x.

Poloni, J.A.T. and Rotta, L.N. (2022) 'Diabetic kidney disease: pathophysiological changes and urinalysis contribution to diagnosis—a narrative review', *Journal of Laboratory and Precision Medicine; Vol 7 (January 30, 2022): Journal of Laboratory and Precision Medicine* [Preprint]. Available at: https://jlpm.amegroups.org/article/view/6743.

Popovici, R. (2022) 'Some data regarding the incidence and susceptibility of different pathogens involved in uti'. Available at: https://doi.org/10.54044/rami.2022.04.03.

Poppelaars, F., Faria, B., da Costa, M.G., Franssen, C.F.M., van Son, W.J., Berger, S.P., Daha, M.R. and Seelen, M.A. (2018) 'The complement system in dialysis: A forgotten story?', *Frontiers in Immunology*, 9(JAN), pp. 1–12. Available at: https://doi.org/10.3389/fimmu.2018.00071.

Poranki, V. and Anvesh kumar, K. (2020) 'A Prospective Study On Therapeutic Management And Outcome Measures In Renal Failure Patients', *International Journal of Scientific and Research Publications (IJSRP)*, 10(8), pp. 194–198. Available at: https://doi.org/10.29322/ijsrp.10.08.2020.p10426.

Portolés, J., Martín, L., Broseta, J.J. and Cases, A. (2021) 'Anemia in Chronic Kidney Disease: From Pathophysiology and Current Treatments, to Future Agents.', *Frontiers in medicine*, 8, p. 642296. Available at: https://doi.org/10.3389/fmed.2021.642296.

Pota, V. and Bell, M. (2024) 'Global Epidemiology and Outcomes of Acute Kidney Injury BT - Nutrition, Metabolism and Kidney Support: A Critical Care Approach', in A. Cotoia, S. De Rosa, F. Ferrari, V. Pota, and M. Umbrello (eds). Cham: Springer Nature Switzerland, pp. 307–317. Available at: https://doi.org/10.1007/978-3-031-66541-7_26.

Pu, C. and Xu, W. (2024) 'Novel Biomarkers for Kidney Disease: New Frontiers in Early Diagnosis and Monitoring', *Academic journal of science and technology*, 12(2), pp. 217–222. Available at: https://doi.org/10.54097/eerw7s17.

Puspitawati, I., P, P.A. and Suromo, L.B. (2018) 'Balance of Proinflammatory and Anti Inflammatory Markers in Routine Hemodialyzed Patients (a Study of End-Stage Renal Failure Patients At the Dr. Sardjito Hospital Yogyakarta)', *Indonesian Journal of Clinical Pathology and Medical Laboratory*, 24(2), pp. 160–164.

Available at: https://doi.org/10.24293/ijcpml.v24i2.1317.

R, N., Kannan, S., Mariappan, T. and P, J. (2017) 'Biochemical evaluation of creatinine and urea in patients with renal failure undergoing hemodialysis.', 1(2), pp. 1–5. Available at: https://www.alliedacademies.org/articles/biochemical-evaluation-of-creatinine-and-urea-in-patients-with-renal-failure-undergoing-hemodialysis.pdf.

Raitanen, M.P., Marttila, T., Nurmi, M., Ala-Opas, M., Nieminen, P., Aine, R. and Tammela, T.L. (2001) 'Human complement factor H related protein test for monitoring bladder cancer.', *The Journal of urology*, 165(2), pp. 374–377. Available at: https://doi.org/10.1097/00005392-200102000-00005.

Ramspek, C.L., Evans, M., Wanner, C., Drechsler, C., Chesnaye, N.C., Szymczak, **M., Krajewska, M., Torino, C., Porto, G., Hayward, S., Caskey, F., Dekker, F.W., Jager, K.J., van Diepen, M. and Investigators, E.S.** (2021) 'Kidney Failure Prediction Models: A Comprehensive External Validation Study in Patients with Advanced CKD.', *Journal of The American Society of Nephrology*, 32(5), pp. 1174–1186. Available at: https://doi.org/10.1681/ASN.2020071077.

Raoofi, S., Pashazadeh Kan, F., Rafiei, S., Hoseinipalangi, Z., Rezaei, S., Ahmadi, S., Masoumi, M., Noorani Mejareh, Z., Roohravan Benis, M., Sharifi, A., Shabaninejad, H., Kiaee, Z.M. and Ghashghaee, A. (2023) 'Hemodialysis and peritoneal dialysis{\textemdash}health-related quality of life: systematic review plus meta-analysis', *BMJ Supportive \& Palliative Care*, 13(4), pp. 365–373. Available at: https://doi.org/10.1136/bmjspcare-2021-003182.

Rasmussen, L.J.H., Petersen, J.E.V. and Eugen-Olsen, J. (2021) 'Soluble Urokinase Plasminogen Activator Receptor (suPAR) as a Biomarker of Systemic Chronic Inflammation', *Frontiers in Immunology*, 12(December), pp. 1–22. Available at: https://doi.org/10.3389/fimmu.2021.780641.

Reisinager, A.C., Niedrist, T., Posch, F., Hatzl, S., Hackl, G., Prattes, J., Schilcher, G., Meißl, A.-M., Raggam, R.B., Herrmann, M. and Eller, P. (2021) 'Soluble urokinase plasminogen activator receptor (suPAR) predicts critical illness and kidney failure in patients admitted to the intensive care unit.', *Scientific Reports*, 11(1), p. 17476. Available at: https://doi.org/10.1038/S41598-021-96352-1.

Renner, B., Laskowski, J., Poppelaars, F., Ferreira, V.P., Blaine, J., Antonioli, A.H., Hannan, J.P., Kovacs, J.M., van Kooten, C., You, Z., Pickering, M.C., Holers, V.M. and Thurman, J.M. (2022) 'Factor H related proteins modulate complement activation on kidney cells', *Kidney International*, 102(6), pp. 1331–

References

1344. Available at: https://doi.org/10.1016/j.kint.2022.07.035.

Ridao, N., Luño, J., de Vinuesa, S.G., Gómez, F., Tejedor, A. and Valderrábano, F. (2001) 'Prevalence of hypertension in renal disease', *Nephrology Dialysis Transplantation*, 16(suppl_1), pp. 70–73. Available at: https://doi.org/10.1093/ndt/16.suppl_1.70.

Rivara, M.B., Ravel, V., Streja, E., Obi, Y., Soohoo, M., Cheung, A.K., Himmelfarb, J., Kalantar-Zadeh, K. and Mehrotra, R. (2018) 'Weekly Standard Kt/Vurea and Clinical Outcomes in Home and In-Center Hemodialysis', *Clinical Journal of the American Society of Nephrology*, 13(3). Available at: https://journals.lww.com/cjasn/fulltext/2018/03000/weekly_standard_kt_vurea_and_clinical_outcomes_in.14.aspx.

Rodelo-Haad, C., Santamaria, R., Muñoz-Castañeda, J.R., Pendón-Ruiz De Mier, M.V., Martin-Malo, A. and Rodriguez, M. (2019) 'FGF23, biomarker or target?', *Toxins*, 11(3). Available at: https://doi.org/10.3390/toxins11030175.

Rodríguez-Ortiz, M.E., Díaz-Tocados, J.M., Muñoz-Castañeda, J.R., Herencia, C., Pineda, C., Martinez-Moreno, J.M., de Oca, A.M., López-Baltanás, R., Alcala-Diaz, J.F., Ortiz, A., Aguilera-Tejero, E., Felsenfeld, A.J., Rodriguez, M. and Almaden, Y. (2020) 'Inflammation both increases and causes resistance to FGF23 in normal and uremic rats.', *Clinical Science*, 134(1), pp. 15–32. Available at: https://doi.org/10.1042/CS20190779.

Rolo, B. (2022) 'Dialysis: A Review of the Mechanisms Behind Complications in Chronic Renal Failure Management Corresponding Author*', *Perspective Journal of Kidney*, 2022(3), p. 17. Available at: https://doi.org/10.35248/2472-1220.22.8.3.17.

Roumeliotis, S., Schurgers, J., Tsalikakis, D.G., D'Arrigo, G., Gori, M., Pitino, A., Leonardis, D., Tripepi, G. and Liakopoulos, V. (2024) 'ROC curve analysis: a useful statistic multi-tool in the research of nephrology', *International Urology and Nephrology*, 56(8), pp. 2651–2658. Available at: https://doi.org/10.1007/s11255-024-04022-8.

Sahin, K. and Yıldıran, H. (2024) 'A Therapeutic Approach in the Management of Chronic Kidney Disease: Plant-Based Dietary Models and Associated Parameters', *Current Nutrition Reports*, 13(1), pp. 39–48. Available at: https://doi.org/10.1007/s13668-023-00515-7.

Sajjad, S.M., Zaman, Y.A., Rahim, M.A., Mahmuda, A., Haque, W. and Uddin, K.N. (2014) 'Parathyroid Hormone Status in Patients with End Stage Renal

References

Disease on Maintenance Haemodialysis', *BIRDEM Medical Journal*, 4(1), pp. 13–17. Available at: https://doi.org/10.3329/BIRDEM.V4I1.18547.

S**amanta, A., Bandyopadhyay, S. and Samanta, D.** (2023) 'Statistical Analyses for Key Risk Factor Identification and Prediction of Chronic Kidney Disease', pp. 1–4. Available at: https://doi.org/10.1109/embc40787.2023.10341104.

Sinawe, H. (2023) Urine Culture. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK557569/ (Accessed: 10 September 2025).

Samarbaf-Zadeh, A.R., Makvandi, M., Hamadi, A., Kaydani, G.A., Absalan, A., Afrough, P., Jahangir, M. and Saeidimehr, S. (2015) 'Prevalence of hepatitis G virus among hemodialysis and kidney transplant patients in Khuzestan Province, Iran', *Jundishapur Journal of Microbiology*, 8(5), pp. 1–5. Available at: https://doi.org/10.5812/jjm.20834.

Sannathimmappa, M.B., Aravindakshan, R., Nambiar, V., Ahmed, W.A., Mazroey, **L.S. Al, Al-Gazali, A.M., Alhooti, A.H., Alhasani, A.B., Blushi, H.Y.A. Al, Risi, E.S.A.A.L. and Maqbali, S. Al** (2024) 'Characterizing Bloodstream Infections in Chronic Hemodialysis Patients: A Single Center Experience', *Preprints* [Preprint]. Available at: https://doi.org/10.20944/preprints202410.0971.v1.

Scherberich, J.E., Fünfstück, R. and Naber, K.G. (2021) 'Urinary tract infections in patients with renal insufficiency and dialysis – epidemiology, pathogenesis, clinical symptoms, diagnosis and treatment', in K.G. Naber and T.E. Bjerklund Johansen (eds) *Urogenital Infections and Inflammations*. Prof. Dr. med. habil. Reinhard Fünfstück Prof. Dr., Sophien-und Hufeland-Klinikum Weimar/Gesundheitszentrum Weimar, Internal medicine, Friedrich-Engels-Strasse 43, 07749 , Jena, Germany, Phone: 0049 3641 396926 / 0049 173 2010424, E-mail: r.fuenfstueck@k: German Medical Science GMS Publishing House. Available at: https://doi.org/10.5680/lhuii000068.

Seiler, S., Heine, G.H. and Fliser, D. (2009) 'Clinical relevance of FGF-23 in chronic kidney disease', *Kidney International*, 76(SUPPL. 114), pp. S34–S42. Available at: https://doi.org/10.1038/ki.2009.405.

Selby, N.M. and Taal, M.W. (2020) 'An updated overview of diabetic nephropathy: Diagnosis, prognosis, treatment goals and latest guidelines', *Diabetes, Obesity and Metabolism*, 22(S1), pp. 3–15. Available at: https://doi.org/10.1111/dom.14007.

References

Sembiring, G.B., Daryanto, B. and Gunawan, A. (2024) 'Profile of End Stage Chronic Kidney Disease due to Obstructive Uropathy Patients Undergoing Hemodialysis', *Brawijaya Journal of Urology*, 5(01), pp. 8–12. Available at: https://doi.org/10.11594/bjurology.2024.005.01.6.

Sepe, V., Libetta, C., Gregorini, M. and Rampino, T. (2022) 'The innate immune system in human kidney inflammaging', *Journal of Nephrology*, 35(2), pp. 381–395. Available at: https://doi.org/10.1007/s40620-021-01153-4.

Shahbazian, H. and Rezaii, I. (2013) 'Diabetic kidney disease; review of the current knowledge', *Journal of renal injury prevention*, 2, pp. 73–80. Available at: https://doi.org/10.12861/jrip.2013.24.

Shamas, N., Khamis, F., Eljaaly, K., Al Salmi, Z. and Al Bahrani, M. (2024) 'Intermittent hemodialysis: a review of the top antimicrobial stewardship practices to be employed', *Antimicrobial Stewardship and Healthcare Epidemiology*, 4(1), pp. 1–8. Available at: https://doi.org/10.1017/ash.2023.525.

Sharif, M., Chitsazian, Z., Moosavian, M., Raygan, F., Nikoueinejad, H., Sharif, A. and Einollahi, B. (2015) 'Immune Disorders in Hemodialysis Patients', *Iranian journal of kidney diseases*, 9, pp. 84–96.

Shen, H., Van Der Kleij, R.M.J.J., Van Der Boog, P.J.M., Chang, X. and Chavannes, N.H. (2019) 'Electronic health self-management interventions for patients with chronic kidney disease: Systematic review of quantitative and qualitative evidence', *Journal of Medical Internet Research*, 21(11). Available at: https://doi.org/10.2196/12384.

Siew, E.D., Peterson, J.F., Eden, S.K., Hung, A.M., Speroff, T., Ikizler, T.A. and Matheny, M.E. (2012) 'Outpatient nephrology referral rates after acute kidney injury.', *Journal of the American Society of Nephrology : JASN*, 23(2), pp. 305–312. Available at: https://doi.org/10.1681/ASN.2011030315.

Singh, R., Chennasamudram, S.P., Sheth, S. and Vasylyeva, T.L. (2014) 'Correlation of Fibroblast Growth Factor 23 with Markers of Inflammation and Endothelial Dysfunction in End-Stage Renal Disease and Type 2 Diabetes Patients on Peritoneal Dialysis', *Journal of diabetes & metabolism*, 5(5), pp. 1–5. Available at: https://doi.org/10.4172/2155-6156.1000371.

Skalec, T., Adamik, B., Kobylinska, K. and Gozdzik, W. (2022) 'Soluble Urokinase-Type Plasminogen Activator Receptor Levels as a Predictor of Kidney Replacement Therapy in Septic Patients with Acute Kidney Injury: An Observational Study', *Journal of Clinical Medicine*, 11(6). Available at:

https://doi.org/10.3390/jcm11061717.

Skinner, S.C., Derebail, V.K., Poulton, C.J., Bunch, D.C., Roy-Chaudhury, P. and Key, N.S. (2021) 'Hemodialysis-Related Complement and Contact Pathway Activation and Cardiovascular Risk: A Narrative Review.', *Kidney medicine*, 3(4), pp. 607–618. Available at: https://doi.org/10.1016/j.xkme.2021.04.006.

Sommerer, C., Zeier, M., Morath, C., Reiser, J., Scharnagl, H., Stojakovic, T., Delgado, G.E., März, W. and Kleber, M.E. (2019) 'Soluble urokinase plasminogen activation receptor and long-term outcomes in persons undergoing coronary angiography', *Scientific Reports*, 9(1), pp. 1–12. Available at: https://doi.org/10.1038/s41598-018-36960-6.

Tahir, A.S., Rasyid, H., Bakri, S., Kasim, H., Harjianti, T., Parewangi, M.L., Iskandar, H., Seweng, A. and Rusman, R.D. (2022) 'Correlation of glomerular filtration rate and fibroblast growth factor-23 levels in chronic kidney disease; sub analysis chronic kidney disease–mineral and bone disorder study', *Journal of nephropharmacology* [Preprint]. Available at: https://doi.org/10.34172/npj.2022.10446.

Tan, K.-K., Chien, T.-W., Kan, W.-C., Wang, C.-Y., Chou, W. and Wang, H.-Y. (2022) 'Research features between Urology and Nephrology authors in articles regarding UTI related to CKD, HD, PD, and renal transplantation.', *Medicine*, 101(41), p. e31052. Available at: https://doi.org/10.1097/MD.0000000000031052.

Tang, P.C.T., Zhang, Y.Y., Chan, M.K.K., Lam, W.W.Y., Chung, J.Y.F., Kang, W., To, K.F., Lan, H.Y. and Tang, P.M.K. (2020) 'The emerging role of innate immunity in chronic kidney diseases', *International Journal of Molecular Sciences*, 21(11), pp. 1–19. Available at: https://doi.org/10.3390/ijms21114018.

Tang, Z. and Sheerin, N. (2009) 'Complement activation and progression of chronic kidney disease', *Hong Kong Journal of Nephrology*, 11(2), pp. 41–46. Available at: https://doi.org/10.1016/S1561-5413(09)60241-6.

Tarchouna, M., Ferjani, A., Ben-Selma, W. and Boukadida, J. (2013) 'Distribution of uropathogenic virulence genes in Escherichia coli isolated from patients with urinary tract infection', *International Journal of Infectious Diseases*, 17(6), pp. e450–e453. Available at: https://doi.org/https://doi.org/10.1016/j.ijid.2013.01.025.

Thajudeen, B., Issa, D. and Roy-Chaudhury, P. (2023) 'Advances in hemodialysis therapy', *Faculty Reviews*, 12(12). Available at: https://doi.org/10.12703/r/12-12.

References

Thi, L. and Nguyen, N. (2020) 'Scholarship & Creative Works @ Digital UNC Stressors and Coping Styles Among Chronic Hemodialysis Patients in Vietnam'.

Thurlow, J.S., Joshi, M., Yan, G., Norris, K.C., Agodoa, L.Y., Yuan, C.M. and Nee, R. (2021) 'Global Epidemiology of End-Stage Kidney Disease and Disparities in Kidney Replacement Therapy.', *American journal of nephrology*, 52(2), pp. 98–107. Available at: https://doi.org/10.1159/000514550.

Thurman, J.M. and Le Quintrec, M. (2016) 'Targeting the complement cascade: novel treatments coming down the pike', *Kidney International*, 90(4), pp. 746–752. Available at: https://doi.org/https://doi.org/10.1016/j.kint.2016.04.018.

Timm, M.R., Russell, S.K. and Hultgren, S.J. (2025) 'Urinary tract infections: pathogenesis, host susceptibility and emerging therapeutics.', *Nature reviews. Microbiology*, 23(2), pp. 72–86. Available at: https://doi.org/10.1038/s41579-024-01092-4.

Torino, C., Pizzini, P., Cutrupi, S., Postorino, M., Tripepi, G., Mallamaci, F., Reiser, J. and Zoccali, C. (2018) 'The soluble receptor for urokinase plasminogen activator (suPAR) serves as a biomarker for innate immunity and inflammation, forecasting cardiovascular and non-cardiovascular events across diverse situations, including type 2 diabetic patients undergoing ', *Kidney international reports*, 3(5), pp. 1100–1109. Available at: https://doi.org/10.1016/j.ekir.2018.05.004.

Trof, R.J., Di Maggio, F., Leemreis, J. and Groeneveld, A.B.J. (2006) 'Biomarkers of acute renal injury and renal failure', *Shock*, 26(3), pp. 245–253. Available at: https://doi.org/10.1097/01.shk.0000225415.5969694.ce.

Valaperta, S., Gamba, S., Leo, C.C.H., Moioli, V., Saiaci, C., Marozzi, R. and Alessio, M. (2024) 'B-070 Supar: what UACR and GFR don't say…', *Clinical Chemistry*, 70(Supplement_1). Available at: https://doi.org/10.1093/clinchem/hvae106.432.

Valdivia, J., Gutiérrez, C., Treto, J., Delgado, E., Méndez, D., Fernández, I., Abdo, A., Pérez, L., Forte, M. and Rodríguez, Y. (2013) 'Prognostic factors in hemodialysis patients: Experience of a Havana Hospital', *MEDICC Review*, 15(3), pp. 11–15. Available at: https://doi.org/10.37757/mr2013v15.n3.4.

Valentini, N., Marchitto, L., Raymond, M., Goyette, G., Kaufmann, D.E., Finzi, A., Suri, R.S. and Lamarche, C. (2022) 'Innate Immunity and SARS-CoV-2 Vaccine Response in Hemodialysis Patients.', *Kidney360*, 3(10), pp. 1763–1768. Available at: https://doi.org/10.34067/KID.0002542022.

Vanholder, R., Gryp, T. and Glorieux, G. (2018) 'Urea and Chronic Kidney

Disease: The Comeback of the Century? (In Uraemia Research)', *Nephrology Dialysis Transplantation*, 33(1), pp. 4–12. Available at: https://doi.org/10.1093/NDT/GFX039.

Vázquez-Sánchez, S., Poveda, J., Navarro-García, J.A., González-Lafuente, L., Rodríguez-Sánchez, E., Ruilope, L.M. and Ruiz-Hurtado, G. (2021) 'An Overview of FGF-23 as a Novel Candidate Biomarker of Cardiovascular Risk.', *Frontiers in physiology*, 12, p. 632260. Available at: https://doi.org/10.3389/fphys.2021.632260.

Velissaris, D., Zareifopoulos, N., Karamouzos, V., Pierrakos, C. and Karanikolas, M. (2022) 'Soluble urokinase plasminogen activator receptor (suPAR) in the emergency department: An update.', *Caspian journal of internal medicine*, 13(4), pp. 650–665. Available at: https://doi.org/10.22088/cjim.13.4.650.

Velissaris, D., Zareifopoulos, N., Koniari, I., Karamouzos, V., Bousis, D., Gerakaris, A., Platanaki, C. and Kounis, N. (2021) 'Soluble Urokinase Plasminogen Activator Receptor as a Diagnostic and Prognostic Biomarker in Cardiac Disease.', *Journal of clinical medicine research*, 13(3), pp. 133–142. Available at: https://doi.org/10.14740/jocmr4459.

Villalon, N., Farzan, N. and Freeman, K. (2018) 'Rate of bacteremia in the hemodialysis patient presenting to the emergency department with fever: a retrospective chart review', *International Journal of Emergency Medicine*, 11(1), pp. 1–6. Available at: https://doi.org/10.1186/s12245-018-0188-5.

Wang, H. (2017) 'Progress in Research on Infection Factors in Hemodialysis Patients', *Infection International*, 5(2), pp. 36–38. Available at: https://doi.org/10.1515/ii-2017-0127.

Wang, Y. and Zhang, Y. (2017) 'Kidney and innate immunity', *Immunology Letters*, 183, pp. 73–78. Available at: https://doi.org/https://doi.org/10.1016/j.imlet.2017.01.011.

Wei, C., El Hindi, S., Li, J., Fornoni, A., Goes, N., Sageshima, J., Maiguel, D., Karumanchi, S.A., Yap, H.-K., Saleem, M., Zhang, Q., Nikolic, B., Chaudhuri, A., Daftarian, P., Salido, E., Torres, A., Salifu, M., Sarwal, M.M., Schaefer, F., Morath, C., Schwenger, V., Zeier, M., Gupta, V., Roth, D., Rastaldi, M.P., Burke, G., Ruiz, P. and Reiser, J. (2011) 'Circulating urokinase receptor as a cause of focal segmental glomerulosclerosis', *Nature Medicine*, 17(8), pp. 952–960. Available at: https://doi.org/10.1038/nm.2411.

Weidemann, D.K., Abraham, A.G., Roem, J.L., Furth, S.L. and Warady, B.A.

References

(2020) ‘Plasma Soluble Urokinase Plasminogen Activator Receptor (suPAR) and CKD Progression in Children.’, *American journal of kidney diseases : the official journal of the National Kidney Foundation*, 76(2), pp. 194–202. Available at: https://doi.org/10.1053/j.ajkd.2019.11.004.

Wen, L., Zhao, Z., Wang, Z., Xiao, J., Birn, H. and Gregersen, J.W. (2019) ‘High levels of urinary complement proteins are associated with chronic renal damage and proximal tubule dysfunction in immunoglobulin A nephropathy’, *Nephrology*, 24(7), pp. 703–710. Available at: https://doi.org/10.1111/nep.13477.

Whitehead, R.D.J., Zhang, M., Sternberg, M.R., Schleicher, R.L., Drammeh, B., Mapango, C. and Pfeiffer, C.M. (2017) ‘Effects of preanalytical factors on hemoglobin measurement: A comparison of two HemoCue® point-of-care analyzers.’, *Clinical biochemistry*, 50(9), pp. 513–520. Available at: https://doi.org/10.1016/j.clinbiochem.2017.04.006.

Williams, E., Bhagani, S. and Harber, M. (2014) ‘Infectious Diseases and the Kidney’, in. Springer, London, pp. 257–268. Available at: https://doi.org/10.1007/978-1-4471-5547-8_24.

Willows, J., Brown, M. and Sheerin, N.S. (2020) ‘The role of complement in kidney disease’, *Clinical Medicine*, 20(2), pp. 156–160. Available at: https://doi.org/https://doi.org/10.7861/clinmed.2019-0452.

Wouk, N. (2021) ‘End-Stage Renal Disease: Medical Management’, *American Family Physician*, 104(5), pp. 493–499.

World Health Organization (2010) WHO guidelines on drawing blood: best practices in phlebotomy. Geneva: World Health Organization. Available at: https://www.ncbi.nlm.nih.gov/books/NBK138665/ (Accessed: 10 September 2025).

Xu, Z., Zhan, H., Zhang, J., Li, Z., Cheng, L., Chen, Q., Guo, Y. and Li, Y. (2025) ‘New biomarkers in IgA nephropathy’, *Clinical Immunology*, 274, p. 110468. Available at: https://doi.org/https://doi.org/10.1016/j.clim.2025.110468.

Yadav, A.K., Kamboj, K., Kundu, M., Ghosh, A., Oommen, J., Gummidi, B., Kumar, V. and Jha, V. (2023) ‘WCN23-0576 SOLUBLE UROKINASE RECEPTOR (suPAR) LEVELS AND INCIDENT CHRONIC KIDNEY DISEASE IN STOP-CKDu COHORT’, *Kidney International Reports*, 8(3, Supplement), pp. S173–S174. Available at: https://doi.org/https://doi.org/10.1016/j.ekir.2023.02.390.

Yamashita, K., Ishiyama, Y., Yoshino, M., Tachibana, H., Toki, D., Konda, R. and Kondo, T. (2022) ‘Urinary Tract Infection in Hemodialysis-Dependent End-Stage Renal Disease Patients.’, *Research and reports in urology*, 14, pp. 7–15.

References

Available at: https://doi.org/10.2147/RRU.S346020.

Yaxley, J. and Yaxley, W. (2023) 'Obstructive uropathy - acute and chronic medical management.', *World journal of nephrology*, 12(1), pp. 1–9. Available at: https://doi.org/10.5527/wjn.v12.i1.1.

Yoshiko, Y., Wang, H., Minamizaki, T., Ijuin, C., Yamamoto, R., Suemune, S., Kozai, K., Tanne, K., Aubin, J.E. and Maeda, N. (2007) 'Mineralized tissue cells are a principal source of FGF23', *Bone*, 40(6), pp. 1565–1573. Available at: https://doi.org/https://doi.org/10.1016/j.bone.2007.01.017.

Younes-Ibrahim, M.S. and Younes-Ibrahim, M. (2022) 'Biomarkers and kidney diseases: a brief narrative review', *Journal of Laboratory and Precision Medicine; Vol 7 (July 30, 2022): Journal of Laboratory and Precision Medicine* [Preprint]. Available at: https://jlpm.amegroups.org/article/view/6914.

Yujie, W. and Jenica, T.M. (2023) 'Life experiences of long-term hemodialysis patients A descriptive review', *Faculty of Health and Occupational Studies* [Preprint].

Zeledon, J.I., McKelvey, R.L., Servilla, K.S., Hofinger, D., Konstantinov, K.N., Kellie, S., Sun, Y., Massie, L., Hartshorne, M.F. and Tzamaloukas, A.H. (2008) 'Glomerulonephritis causing acute renal failure during the course of bacterial infections. Histological varieties, potential pathogenetic pathways and treatment', *International Urology and Nephrology*, 40(2), pp. 461–470. Available at: https://doi.org/10.1007/S11255-007-9323-6.

Zeng, D., Zha, A., Lei, Y., Yu, Z., Cao, R., Li, L., Song, Z., Li, W., Li, Y., Liu, H., Huang, S., Dong, X., Krämer, B., Hocher, B., Yin, L., Yun, C., Morgera, S., Guan, B., Meng, Y., Liu, F., Hu, B. and Luan, S. (2023) 'Correlation of Serum FGF23 and Chronic Kidney Disease-Mineral and Bone Abnormality Markers with Cardiac Structure Changes in Maintenance Hemodialysis Patients', *Evidence-based Complementary and Alternative Medicine*, 2023. Available at: https://doi.org/10.1155/2023/6243771.

Zhan, Z.-S., Shi, J., Zheng, Z.-S., Zhu, X.-X., Chen, J., Zhou, X.-Y. and Zhang, S.-Y. (2024) 'Epidemiological insights into seasonal, sex-specific and age-related distribution of bacterial pathogens in urinary tract infections', *Exp Ther Med*, 27(4), p. 140. Available at: https://doi.org/10.3892/etm.2024.12428.

Zhang, J.-J., Jiang, L., Liu, G., Wang, S.-X., Zou, W.-Z., Zhang, H. and Zhao, M.-H. (2009) 'Levels of urinary complement factor H in patients with IgA nephropathy are closely associated with disease activity.', *Scandinavian journal of*

References

immunology, 69(5), pp. 457–464. Available at: https://doi.org/10.1111/j.1365-3083.2009.02234.x.

Zhang, L. and Qin, W. (2023) 'Research progress of fibroblast growth factor 23 in acute kidney injury.', *Pediatric nephrology (Berlin, Germany)*, 38(7), pp. 2013–2022. Available at: https://doi.org/10.1007/s00467-022-05791-z.

Zhang, Wenwen, Gu, Y., Zhou, J., Wang, J., Zhao, X., Deng, X., Li, H., Yan, L., Jiao, X. and Shao, F. (2024) 'Clinical value of soluble urokinase-type plasminogen activator receptor in predicting sepsis-associated acute kidney injury', *Renal Failure*, 46(1), p. Available at: https://doi.org/10.1080/0886022X.2024.2307959.

Zhang, Wei, Wang, Y., Wang, K., Li, J., Liu, J., Li, S., Song, L., Liao, C., Yang, X., Li, P. and Liu, X. (2024) 'Hybrid Sequencing-Based Genomic Analysis of Klebsiella pneumoniae from Urinary Tract Infections Among Inpatients at a Tertiary Hospital in Beijing', *Infection and Drug Resistance*, 17(March), pp. 1447–1457. Available at: https://doi.org/10.2147/IDR.S448253.

Zhou, L.W., Liu, Q.Z. and Yang, S.H. (2024) 'Advances in the construction of risk prediction models for chronic kidney failure', 58(5), pp. 690–697. Available at: https://doi.org/10.3760/cma.j.cn112150-20230814-00091.

Zimmerman, T. (2022) 'Immune System Dysfunction and Inflammation in Hemodialysis Patients: Two Sides of the Same Coin.', *Journal of clinical medicine*, 11(13). Available at: https://doi.org/10.3390/jcm11133759.

Appendices

Appendices 1

Questionnaire

Case No.
Date:

Hospital name:

Patient name:-

Age: -

Sex:-

Weight: - Kg

Height: - cm

Telephone number:-

Profession:Residence:-

Duration of disease:-

Final diagnosis of the disease:-

Other diseases:-

Type of Treatment: -

Smoking:-

Laboratory tests:

Appendices 2

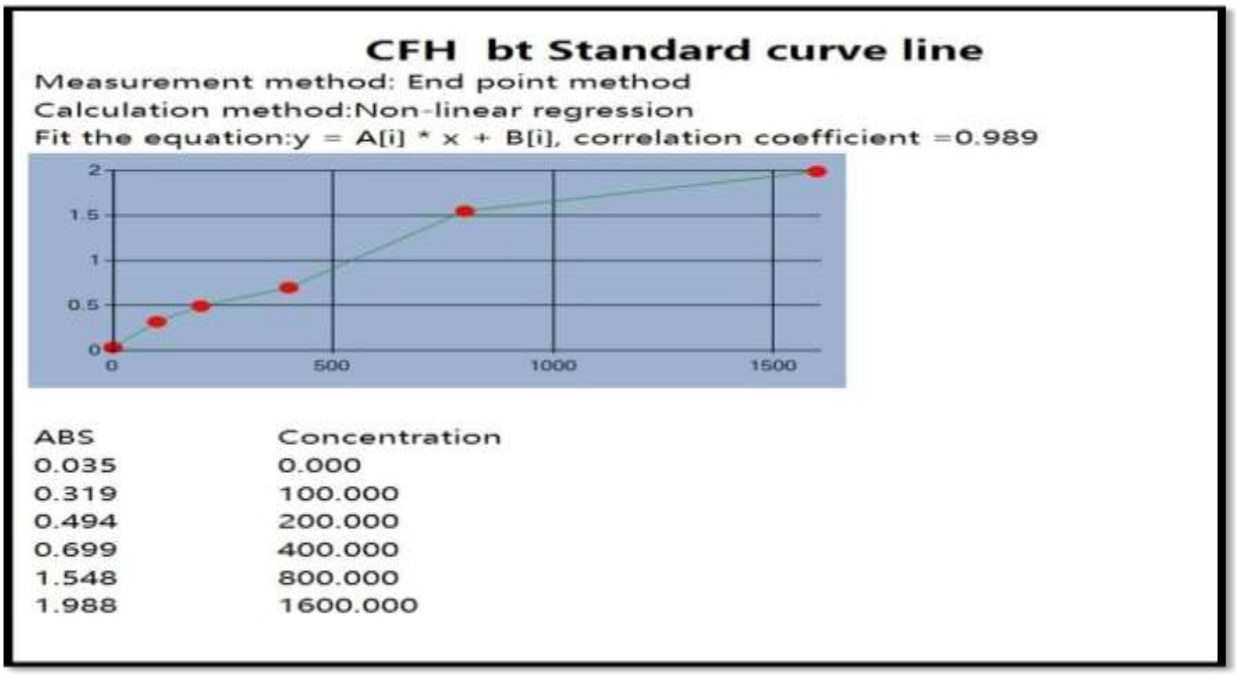

Figure 2: The standard curve of CFH

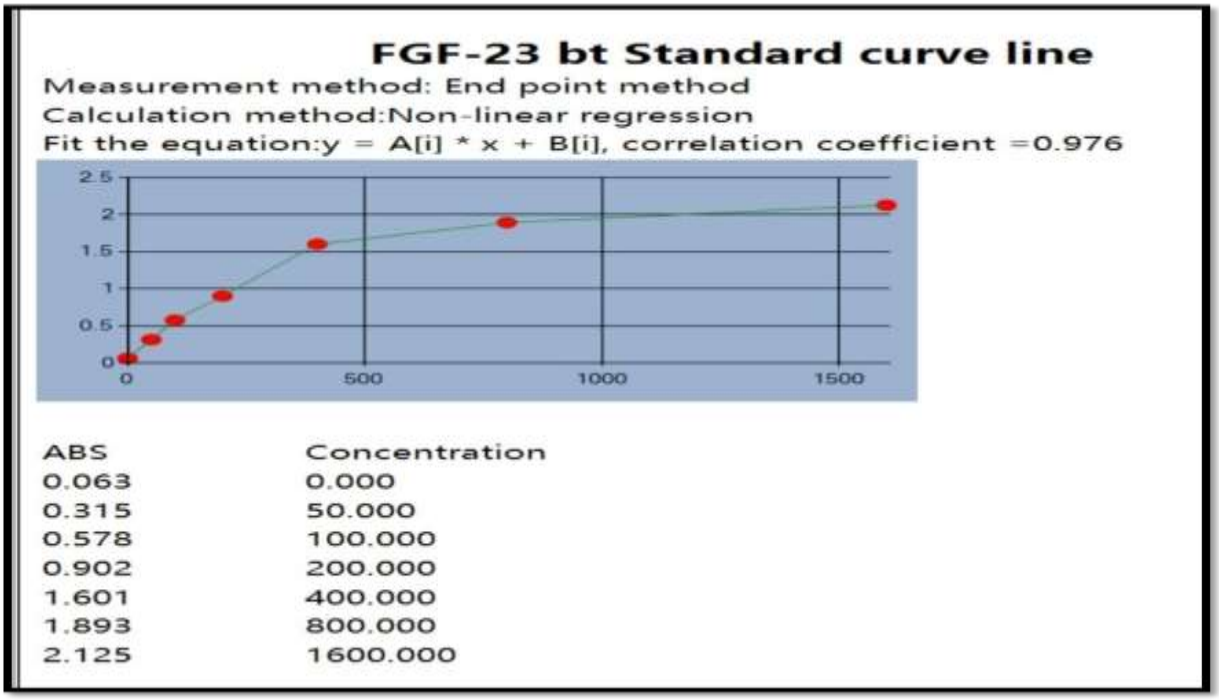

Figure 3: The standard curve of FGF23

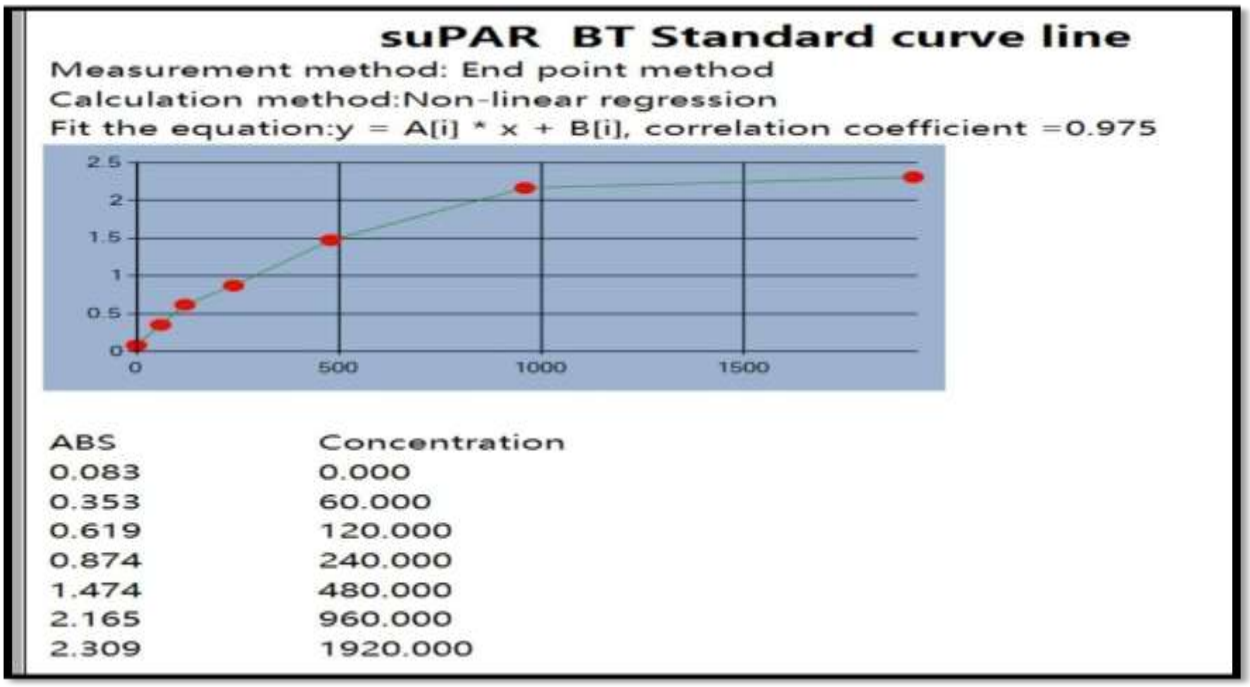

Figure 3: The standard curve of suPAR

Appendices 3

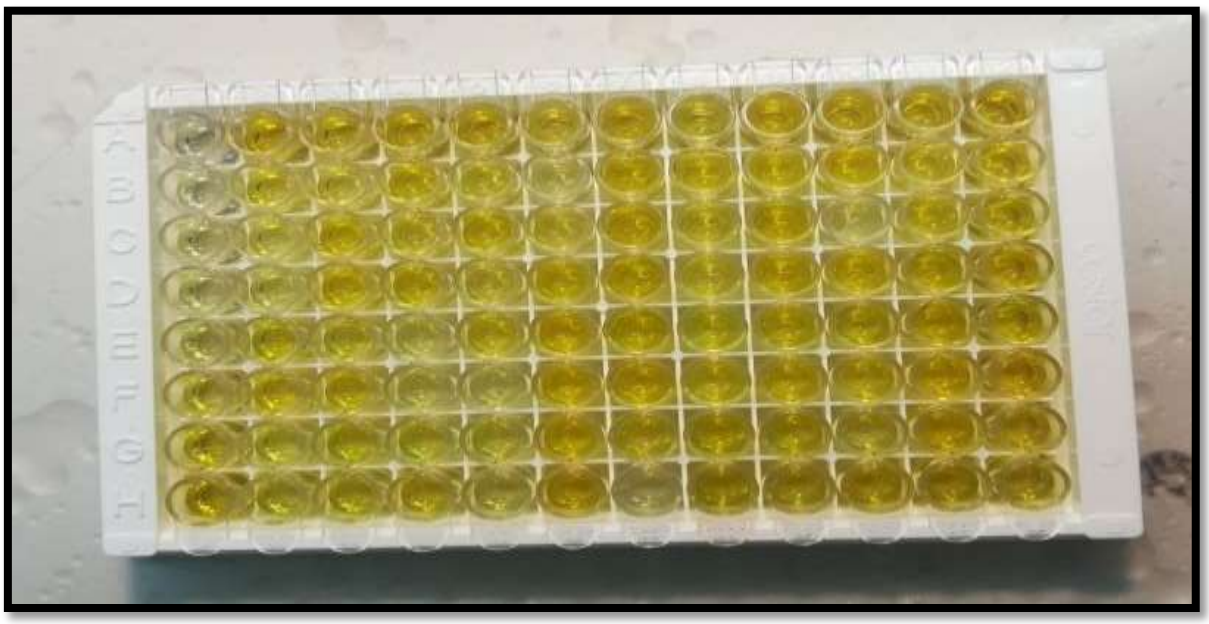

Figure (4): Microtiter plate final reaction (after adding the stop solution).

Figure 5: ELISA Kits for CFH,suPAR and FG 1

Appendices 4

Health Department
Training and Human Development Center
Research & Knowledge Management Unit

جمهورية العراق

وزارة الصحة
دائرة صحة كربلاء المقدسة
مركز التدريب والتنمية البشرية
وحدة ادارة البحوث والمعرفة

العدد: ٧٧٠
التاريخ: ٣ / ١١ / ٢٠٢٤

الى/ جامعة كربلاء-كلية الطب
م/ تسهيل مهمة

تحية طيبة....
نود إعلامكم بأنه لا مانع لدينا من تسهيل مهمة الطالب (احمد حسن كسار حميدي) لانجاز بحثه الموسوم:
((Role of hemodialysis on some immunological markers and bacterial infections in renal failure patients))
في مؤسستنا الصحية مدينة الامام الحسين (ع) الطبية وباشراف الدكتور (حسنين صلاح الخياط) على ان لا تتحمل دائرتنا اي نفقات مادية مع الاحترام .

الدكتورة
تقوى خضر عبد الكريم
مدير مركز التدريب والتنمية البشرية
٣ / ١١ / ٢٠٢٤

نسخة منه الى
مدينة الامام الحسين (ع) الطبية /لإجراء اللازم مع الاحترام . .
مركز التدريب والتنمية البشرية /وحدة ادارة البحوث والمعرفة / مع الاوليات .

الخلاصة

تُعد العدوى من المضاعفات الشائعة والخطيرة لدى مرضى غسيل الكلى، حيث تُصنّف كثاني أهم سبب للوفاة في هذه الفئة من المرضى. يؤدي الفشل الكلوي المزمن إلى إضعاف الجهاز المناعي، مما يزيد من قابلية الجسم للإصابة بالعدوى، لا سيما عدوى المسالك البولية، التي تُعد من أكثر أنواع العدوى شيوعًا في هذا المرض . وقد أظهرت دراسات حديثة وجود علاقة بين بعض المؤشرات الحيوية مثل عامل نمو الخلايا الليفية 23(FGF23) ، وعامل المتمم(CFH) H ، والمستقبل المنشط لليوروكيناز البلازمينوجين القابل للذوبان (suPAR)، وبين تدهور وظائف الكلى والتغيرات المناعية المصاحبة لها.

هدفت هذه الدراسة إلى تحديد وتوزيع أنواع العدوى البكتيرية المسببة لعدوى المسالك البولية لدى مرضى غسيل الكلى، إضافةً إلى تقييم مستويات بعض المؤشرات المناعية(FGF23، CFH، suPARو) ومقارنتها بين المرضى المصابين بالفشل الكلوي مع أو بدون عدوى، وبين مرضى عدوى المسالك البولية فقط، ومجموعة سيطرة سليمة. وقد أجريت هذه الدراسة في مركز غسيل الكلى بمستشفى الإمام الحسين (ع) في محافظة كربلاء، خلال الفترة الممتدة من أكتوبر 2024 إلى يناير 2025. شملت الدراسة جمع 120 عينة دم، قُسّمت إلى أربع مجموعات: 30 مريضًا بالفشل الكلوي المصحوب بعدوى المسالك البولية، و30 مريضًا بالفشل الكلوي بدون عدوى، و30 مريضًا بعدوى مسالك بولية فقط، و30 فردًا كمجموعة ضابطة سليمة. تراوحت أعمار المشاركين بين 10 و80 عامًا.

تم إجراء مزرعة بول لعزل البكتيريا المسببة للعدوى وتحديد أنواعها، كما تم جمع عينات دم لقياس المؤشرات المناعيةFGF23 ، CFH، suPAR، إضافة إلى تقييم وظائف الكلى (اليوريا، الكرياتينين)، وتحاليل الدم، ومستويات الإلكتروليتات، وعدد من الهرمونات مثل هرمون الغدة الجار درقية.

أظهرت نتائج الدراسة الحالية أن E. coli كانت أكثر البكتريا شيوعًا، حيث بلغت 30 عزلة (50.00%)، تليها Klebsiella pneumonia بعشر عزلات (16.67%)، ثم Staphylococcus aureus بسبع عزلات (11.67%). وشملت مسببات الأمراض الأخرى المُحددة: Enterococcus faecalis (3 عزلات؛ 5.00%)، وpseudomonas aeruginosa (3 عزلات؛ 5.00%)، و proteus mirabilis (3 عزلات؛ 5.00%). بالإضافة إلى ذلك، تم حددت عزلات مفردة (عزلة واحدة لكل نوع؛ 1.67%) *Staphylococcus haemolyticus, Staphylococcus hominins, staphylococcus saprophyticus*

كما أظهرت الدراسة وجود فروق معنوية ذات دلالة إحصائية ($P < 0.05$) في مستويات المؤشرات الحيوية FGF23، CFH، suPARبين المجموعات المختلفة، حيث كانت هذه المستويات مرتفعة بشكل ملحوظ لدى مرضى الفشل الكلوي، سواء المصابين أو غير المصابين بعدوى المسالك البولية، مقارنةً بالمجموعات

الأخرى. وُجد أيضًا ارتفاع في مستويات اليوريا، الكرياتينين، الفوسفور، الفيريتين، وهرمون الغدة الجار درقية لدى مرضى الفشل الكلوي. كما كشفت التحاليل الإحصائية عن وجود علاقات ارتباط إيجابية بين FGF23وCFH (r = 0.768، p < 0.001)، وبين FGF23 وsuPAR (r = 0.674، p < 0.001)، وبين CFH وsuPAR (r = 0.670، p < 0.001). كما وُجد ارتباط إيجابي بين FGF23 ومستوى الفوسفور في مصل الدم(r = 0.342 ، p = 0.008).

من خلال هذه النتائج، تبين أن النساء أكثر عرضة للإصابة بعدوى المسالك البولية مقارنة بالرجال، وأن E. coliكانت البكتيريا الأكثر شيوعًا. كما تُبرز النتائج أهمية متابعة المؤشرات المناعيةFGF23 ، CFH، suPARلدى مرضى الفشل الكلوي، لما لها من دور في الكشف المبكر عن التدهور في الوظائف الكلوية، وبالتالي تمكين التدخل العلاجي المناسب في الوقت المناسب للحد من تفاقم المرض والمضاعفات المصاحبة له. إضافةً إلى ذلك، فإن فهم الجوانب الديموغرافية المرتبطة بعدوى المسالك البولية يُسهم في وضع استراتيجيات وقائية موجهة، خاصة بين الإناث الأصغر سنًا، بهدف تقليل معدلات الإصابة والمضاعفات الكلوية الناتجة عنها.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الطب
فرع الأحياء المجهرية

دراسة البكتيريا المسببة لالتهابات المسالك البولية والعلامات المناعية الانتقائية suPAR , CFHو FGF23 لدى مرضى الغسيل الكلوي المصابين بالفشل الكلوي

رسالة

مقدمة الى مجلس كلية الطب/جامعة كربلاء كجزء من متطلبات نيل درجة الماجستير في الاحياء المجهرية الطبية

من قبل الطالب

احمد حسن كسار حميدي

بكالوريوس علوم حياه/ كلية العلوم /جامعة القادسية

بأشراف

أ.د علي جليل علي الياسري

أ.م.د مسار رياض رشيد الموسوي

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