THE CORRELATION STUDY BETWEEN CYTOKINES IL-6, TNF- α AND OTHER BIOCHEMICAL PARAMETERS WITH ADIPOKINES VISFATIN AND CHEMERIN IN END STAGE RENAL DISEASE PATIENTS UNDERGOING HAEMODIALYSIS

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by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

%	Percent
+	Plus
<	Less than
=	Equals
>	Greater than
±	Plus-Minus
≤	Less than or equal to
µkat	Microkatals
µL	Microliter
dL	Deciliter
gm	Gram
IU	International Unit
Kg	Kilogram
L	Liter
mg	Milligram
ml	Milliliter
mmol	Millimole
µmol	Micromole

LIST OF ABBREVIATIONS

AKI	Acute kidney in
A	Absorbance
ARF	Acute Renal Failure
ALT	Alanine transaminase
Alb	Albumin
ALkP	Alkaline phosphatase
AST	Aspartate aminotransferase
BMI	Body Mass Index
Ca^{+2}	Calcium
CVD	Cardiovascular disease
CMS	Centers for medicare & mediacaid services
Cl^{-}	Chloride
CETP	Cholesteryl Ester Transfer Protein
CKD	Chronic kidney disease
CRF	Chronic renal failure
CRD	Chronic renal disease
CSF	Colony-stimulating factors
Ccr	Creatinine clearance rate
CPC	Cresolphthalein Complexone
DM	Diabetes mellitus
DGAT	Diacylglycerol Acyltransferase
BililD	Direct Bilirubin

ESRD	End-session renal disease
ELISA	Enzyme-linked immunosorbent assay
GFR	Glomerular filtration rate
GPT	Glutamate Pyruvate Transaminase
GOT	Glutamic Oxaloacetic Transaminase
HDF	Hemodiafiltration
HD	Haemodialysis
HF	Hemofiltration
HDL	High-density lipoprotein
HRP	Horseradish peroxidase
HT	Hypertension
ICU	Intensive care unit
IL-6	Interleukin 6
IL-1	Interleukin 1
KDOQI	Kidney Disease Outcomes Quality Initiative
LDH	Lactate Dehydrogenase
LCAT	Lecithin Cholesterol Acyl Transferase
LPL	Lipoprotein Lipase
MHD	Maintenance Haemodialysis
MCO	Medium cut off
OD	Optical density
PTH	Parathyroid hormone

PBS	Phosphate buffered Saline
K^+	Potassium
LRP	Receptor-Related Protein
RRT	Renal replacement therapy
Na^+	Sodium
TMB	Tetramethylbenzidine
BililT	Total Bilirubin
TC	Total cholesterol
TP	Total protein
Tg	Triglycerides
TBS	Tris buffered Saline
TNF-α	Tumor necrosis factor alpha
UV	Ultraviolet
UA	Uric acid
UTI	Urinary tract infections
VLDL	Very Low-Density Lipoprotein

LIST OF APPENDICES

KAJIAN KOLERASI ANTARA SITOKIN IL-6, TNF- α, DAN PARAMETER BIOKIMIA DENGAN ADIPOKIN VISFATIN DAN KEMERIN PADA PESAKIT RENAL PERINGKAT AKHIR YANG MENJALANI HEMODIALISIS

ABSTRAK

Penyakit buah pinggang tahap akhir (PBPA) merupakan tahap terakhir penyakit buah pinggang kronik (PBK) yang dicirikan oleh kehilangan fungsi buah pinggang, memerlukan dialisis atau pemindahan buah pinggang untuk mengekalkan hidup. Visfatin dan kemerin adalah adipokin yang dipercayai terlibat dalam perkembangan penyakit kardiovaskular, suatu komplikasi utama PBK. Tahap tinggi visfatin dan kemerin telah dikaitkan dengan peningkatan radang, tekanan oksidatif, dan disfungsi endotelia dalam pesakit PBK. Oleh itu, menilai tahap visfatin dan kemerin mungkin bermakna dalam meramal kemajuan PBK dan hubungannya dengan penyakit kardiovaskular aterosklerotik. Kajian ini bertujuan untuk menentukan tahap pelbagai parameter pada pesakit PBPA yang menjalani hemodialisis, menilai bagaimana dialisis mempengaruhi parameter ini semasa sesi hemodialisis, dan menghubungkan tahap adipokin, penanda keradangan, dan parameter metabolik pada pesakit PBPA. Kajian ini mengukur sitokin serum (TNF-α, IL-6), adipokin serum (visfatin, chemerin), vitamin D, amilase, glukosa, urea, profil lipid serum (kolesterol, HDL, TG), ujian fungsi hati serum (ALT, AST, ALP, bilirubin langsung, bilirubin total, protein total, albumin, LDH), dan ujian fungsi buah pinggang serum (asid urik, kreatinin, natrium, kalium, klorida, kalsium). Kajian melibatkan 100 pesakit dengan penyakit buah pinggang tahap akhir (PBPA) dari unit dialisis Hospital Pengajaran Ramadi, Iraq. Pesakit ini menjalani tiga sesi hemodialisis, dan pengukuran

diambil sebelum dan selepas setiap sesi dialisis. Selain itu, satu kumpulan kawalan yang terdiri daripada 100 individu yang kelihatan sihat juga disertakan. Pada pesakit PBPA, terdapat peningkatan keradangan, disregulasi metabolik, dan perubahan fungsi hati dengan peningkatan IL-6, TNF-α, chemerin, visfatin, kolesterol, TG, amilase, dan APL. Penurunan yang signifikan diperhatikan dalam vitamin D, albumin, HDL, Ca+2, Na+, AST, ALT, dan bilirubin total berbanding dengan kumpulan yang sihat. Membran dialisis mempengaruhi parameter secara berbeza, meningkatkan LDH tetapi mengurangkan UA, K+, urea, dan Cl-. Dialisis juga mengurangkan tahap kreatinin dan glukosa yang tinggi, menunjukkan penyingkiran produk buangan yang berkesan. Korelasi positif ditemui di antara penanda keradangan, sementara vitamin D menunjukkan korelasi negatif yang signifikan, menunjukkan peranan perlindungan yang berpotensi terhadap keradangan dalam PBPA. Kesimpulannya, kajian menunjukkan bahawa tahap tinggi sitokin serum, termasuk chemerin, visfatin, IL-6, dan TNF-α, bersamaan dengan dislipidemia dan ketidakseimbangan elektrolit dalam pesakit PBPA yang menjalani dialisis, mungkin memainkan peranan dalam memajukan penyakit buah pinggang dan mempercepat timbulnya komplikasi kardiovaskular aterosklerotik. Kajian berpanjangan untuk menjejak perubahan dalam tahap sitokin dan parameter lain dalam jangka masa yang lebih lama pada pesakit PBPA boleh dijalankan. Ini boleh memberikan pandangan tentang sifat dinamik penanda biologi ini dan hubungan mereka dengan kemajuan penyakit buah pinggang kronik.

THE CORRELATION STUDY BETWEEN CYTOKINES IL-6, TNF- α AND OTHER BIOCHEMICAL PARAMETERS WITH ADIPOKINES VISFATIN AND CHEMERIN IN END STAGE RENAL DISEASE PATIENTS UNDERGOING HAEMODIALYSIS

ABSTRACT

End-stage renal disease (ESRD) is the final stage of chronic kidney disease (CKD) characterized by the loss of kidney function, necessitating dialysis or kidney transplantation to sustain life. Visfatin and chemerin are adipokines believed to be involved in the development of cardiovascular disease, a major complication of CKD. Elevated levels of visfatin and chemerin have been associated with increased inflammation, oxidative stress, and endothelial dysfunction in CKD patients. Therefore, assessing visfatin and chemerin levels may be valuable in predicting CKD progression and its association with atherosclerotic cardiovascular disease. This study aimed to determine the levels of various parameters in patients with ESRD undergoing haemodialysis, to evaluate how dialysis affect these parameters during haemodialysis sessions and to correlate the levels adipokines, inflammatory markers and metabolic parameters in ESRD patients. The study measured serum cytokines (TNF-α, IL-6), serum adipokines (visfatin, chemerin) vitamin D, amylase, glucose, urea, serum lipid profile (cholesterol, HDL, TG), serum liver function test (ALT, AST, ALP, direct bilirubin, total bilirubin, total protein, albumin, LDH) and serum renal function test (uric acid, creatinine, sodium, potassium, chloride, calcium). The research involved 100 patients with end-stage renal disease (ESRD) from the dialysis unit at Ramadi Teaching Hospital, Iraq. These patients underwent three haemodialysis sessions,

and measurements were taken both before and after each dialysis session. Additionally, a control cohort comprising 100 apparently healthy individuals was incorporated. In ESRD patients, heightened inflammation, metabolic dysregulation, and liver function alterations were observed with elevated IL-6, TNF-α, chemerin, visfatin, cholesterol, TG, amylase, and APL. Significant decreases were noted in vitamin D, albumin, HDL, Ca+2, Na+, AST, ALT, and total bilirubin compared to the healthy group. Dialysis membranes affected parameters differently, increasing LDH but decreasing UA, K+, urea, and Cl-. Dialysis also reduced elevated creatinine and glucose levels, indicating effective waste product removal. Positive correlations were found among inflammatory markers, while vitamin D showed a significant negative correlation, suggesting a potential protective role against inflammation in ESRD. In conclusion, the study indicates that elevated levels of serum cytokines, including circulating chemerin, visfatin, IL-6, and TNF-α, coupled with dyslipidaemia and electrolyte imbalances in end-stage renal disease (ESRD) patients undergoing dialysis, may play a role in advancing renal disease and hastening the onset of atherosclerotic cardiovascular complications. Longitudinal studies to track the changes in cytokine levels and other parameters over an extended period in ESRD patients can be conducted. This could provide insights into the dynamic nature of these biomarkers and their relationship with chronic kidney disease progression.

CHAPTER 1

INTRODUCTION

1.1 Renal Functions

The urinary system is composed of the kidneys, ureters, urinary bladder, and urethra. The functional units of the kidneys are called nephrons, which are composed of tubules, glomerulus, and collecting ducts. The responsibility of the kidneys is to remove waste products from the blood and maintain the pH balance and salt levels in the body. This vital process results in the formation of urine. In addition to waste removal, the kidneys also play a role in regulating blood pressure, producing red blood cells, and balancing acid-base levels. Moreover, kidneys metabolize bones by converting vitamin D to its active form, maintaining calcium/phosphate balance. Renal failure can have a detrimental impact on all of these functions (Breshears & Confer, 2017)**.**

1.2 Chronic Kidney Disease (CKD)

Chronic kidney disease (CKD) or also called chronic renal failure (CRF), is a progressive decrease of renal function that necessitates long-term treatment with renal replacement therapy. Haemodialysis is a type of kidney replacement therapy in which the body's waste products such as urea, creatinine and excess water are eliminated. Kidney function declines slowly and steadily as a result of CRF (Vaidya & Aeddula, 2021). Renal clearance, also known as glomerular filtration rate (GFR), decreases with time in CRF, resulting in the accumulation of creatinine, urea and other substances in the blood. CKD refers to a condition in which the kidneys' function and/or structure are abnormal. Regardless of a clinical diagnosis, CKD is defined as having either kidney injury

(albuminuria) or dysfunction renal function (GFR< 60 ml/min per 1.73 m^2) over a period of three months or more. There is evidence that medication can minimize or avoid the development of complications, prevent or reduce the progression of CKD, and also reduce the risk of cardiovascular disease (Webster *et al.,* 2017).

1.2.1 Incidence and Prevalence of Chronic kidney disease

Chronic kidney disease (CKD) is a significant health issue worldwide, with a substantial impact on public health systems and individuals' quality of life. Understanding the epidemiology of CKD is crucial for developing effective prevention and management strategies.

Globally, CKD has become a growing public health concern. According to the World Health Organization (WHO), an estimated 10% of the global population is affected by CKD (Luyckx *et al.*, 2018). The prevalence varies among different regions and countries, influenced by several factors such as demographics, socioeconomic status, cultural practices, and healthcare infrastructure. In recent years, there has been an alarming rise in the incidence and prevalence of CKD, primarily due to the increasing prevalence of risk factors such as diabetes, hypertension, and obesity (Hill *et al.*, 2016).

In Iraq, CKD is a significant health problem. The country has experienced an increase in the incidence and prevalence of CKD, posing substantial challenges to its healthcare system. Several factors contribute to the burden of CKD in Iraq, including a high prevalence of diabetes and hypertension, exposure to environmental toxins such as heavy metals in the water supply, inadequate access to healthcare services, and limited awareness about kidney disease prevention and management. Additionally, the ongoing

conflict and instability in the region further exacerbate the challenges faced in addressing CKD effectively (Ali, 2018; Majeed *et al.,* 2018).

In the surrounding region, similar trends in CKD epidemiology are observed. Countries such as Iran, Saudi Arabia, and Jordan also report high prevalence rates of CKD, primarily attributed to the rising incidence of diabetes and hypertension. Additionally, the high prevalence of consanguineous marriages in some communities contributes to genetic predisposition and an increased risk of hereditary kidney diseases (Alaraj *et al.,* 2017; Tabrizi *et al.,* 2016).

On a global scale, efforts are being made to tackle the burden of CKD. International organizations, including the World Health Organization and the International Society of Nephrology, have recognized CKD as a priority and are actively promoting strategies for prevention, early detection, and management. These initiatives aim to raise awareness, improve healthcare infrastructure, enhance screening programs, and ensure access to appropriate treatments, including renal replacement therapy such as dialysis and kidney transplantation (Hill *et al.,* 2016; Luyckx *et al.,* 2018).

1.2.2 End-stage of Renal Disease (ESRD)

End-stage renal disease (ESRD) is defined as a GFR of less than 15 ml/min/1.73m^2 and requires renal replacement therapy (dialysis or kidney transplantation). Acute and chronic cardiovascular illness, other comorbidities, and death are all increased risks for ESRD patients. In this stage, treatment modality is provided (haemodialysis or peritoneal dialysis) and patients are evaluated for transplantation (Al-Obaidy, 2017; Vaidya & Aeddula, 2021).

1.2.3 Haemodialysis as a Renal Replacement Therapy

Dialysis is a life-sustaining treatment for individuals with chronic kidney failure. It is a process that helps to remove waste products and excess fluids from the body when the kidneys are no longer able to perform these functions adequately. There are two main types of dialysis: haemodialysis and peritoneal dialysis. Haemodialysis involves using a machine to filter the blood outside the body, while peritoneal dialysis involves using the lining of the abdomen to filter the blood inside the body. In both types of dialysis, a specialized filter is used to remove waste products and excess fluids from the blood (Andreoli & Totoli, 2020; Tanaka *et al.,* 2020). These filters, known as dialyzers, are made up of semipermeable membranes that allow small molecules to pass through while retaining larger molecules like proteins and blood cells. One of the main challenges of dialysis is maintaining the proper balance of electrolytes in the body, such as sodium, potassium, and calcium (Kandi *et al.,* 2022; Westphalen *et al.,* 2022).

Dialysis can cause significant shifts in electrolyte levels, which can lead to complications like low blood pressure, muscle cramps, and arrhythmias. In addition to electrolyte imbalances, dialysis can also affect the levels of other substances in the blood, such as glucose, bicarbonate, and albumin. Despite these challenges, dialysis is a critical treatment for individuals with chronic kidney failure. It can help to improve quality of life, prolong survival, and prevent complications associated with kidney failure, such as cardiovascular disease, bone disease, and neurological symptoms. However, ongoing research is needed to improve the safety and effectiveness of dialysis and to develop new treatments for chronic kidney failure (Cozzolino *et al.,* 2018; Tsai *et al.,* 2020a; Young, S. 2021).

1.3 Contributing Factors in the Pathogenesis of Kidney Disorders

The most common causes of chronic kidney failure are diabetes and high blood pressure. Therefore, there is a growing proportion of patients with diabetic nephropathy and hypertensive nephropathy. CKD can be classified into five stages according to the level of GFR. In stage five, also known as end-stage renal disease (ESRD), the level of GFR is less than 15 ml/min per 1.73 m^2, which requires renal replacement therapy such as dialysis or renal transplantation (Trevisani *et al.*, 2021).

Hypertension leads to damage of the blood vessels in the kidneys, affecting the excretion of waste products. Waste may be excreted into the extracellular fluid, further raising blood pressure and eventually leading to end-stage renal failure. Over time, CKD causes a multitude of complications, cerebrovascular and peripheral vascular disorders, as well as cardiovascular diseases, are the most frequent. In ESRD patients, cardiovascular disease (CVD) is the primary cause of morbidity and mortality, and it can occur even in the early stages of the disease, even without visible vascular disease. The risk of cardiovascular mortality in dialysis patients is significantly higher than in an age-matched general population, with CVD being the primary cause of death among dialysis patients (Cozzolino *et al*., 2018).

1.4 Role of Adipokines

Adipose tissue is now recognized as an active endocrine organ that secretes adipokines, which are a type of inflammatory cytokine that interferes with insulin sensitivity, lipid and glucose metabolism, and also contributes to the inflammatory process. Adipokines play a role in the development of renal and cardiovascular problems. In kidney injury, adipokines induce inflammation, generate oxidative stress, regulate endothelial dysfunction, and promote renal sympathetic nerve activity. This can lead to decreased cancellous bone mass but increased cortical bone mass. Adipokines may also contribute to the development of renal anemia.

Moreover, adipokines may potentially contribute to the development of cardiovascular complications and atherosclerosis. Recent studies have shown that circulating adipokines are associated with the development of coronary artery disorders, including acute myocardial infarction (AMI) and unstable angina pectoris (UAP) (Vahdat, 2018).

1.4.1 Serum Chemerin

Chemerin is a newly discovered adipocytokine that has been demonstrated to influence adipocyte development, modulate adipocyte gene expression, and have a role in the etiology of nephropathy. Chemerin levels in the blood were found in a recent study to have a substantial positive relationship with indicators of dyslipidaemia and inflammation. Chemerin, which regulates innate immune cell function, is discovered to be highly expressed in adipose tissue, innate immune system cells, and the liver (Tahoun, 2019). As a result, Chemerin could be a link between fat and inflammation, as well as a player in the

development of cardiovascular complications and atherosclerosis. Therefore, recent reports showed that the circulating adipokines were linked to the occurrence of coronary artery disorders, such as acute myocardial infarction (AMI) and unstable angina pectoris (UAP) (Kaur *et al*., 2018).

1.4.2 Serum Visfatin

Visfatin, is a novel adipokine that is identified as a ubiquitous adipokine first described by Fukuhara *et al*., (2005). The rise levels of Visfatin in patients with ESRD have been reported to significantly increase and be correlated with endothelial dysfunction. Thus, Visfatin may be associated with the progression of atherosclerosis Duman *et al*., (2019) found a close association between Visfatin and Cardiovascular disease CVD.

1.5 Potential Biomarkers in Chronic Kidney Disease

In chronic kidney disease (CKD), the identification and evaluation of potential biomarkers play a crucial role in understanding disease progression and guiding appropriate therapeutic interventions. Among the various factors influencing CKD, inflammation has emerged as a key contributor to the pathogenesis and complications of the disease. Therefore, investigating the role of potential biomarkers, particularly those associated with inflammation, becomes paramount in advancing the understanding of CKD ((Thaha & Widiana, 2019).

1.5.1 Interleukin- 6

Interleukin 6 (IL-6) is a pleiotropic cytokine that has a role in a variety of biological processes. The inflammatory response is orchestrated by a number of pro-inflammatory (TNF-α, and interleukin-1) and anti-inflammatory (interleukin-10) cytokines. The information at hand points to IL-6 and its soluble receptor sIL-6R, as the central regulators of the inflammatory response. The IL-6 system stimulates inflammatory processes by stimulating lymphocyte activation and proliferation, B-cell differentiation, leukocyte recruitment, and liver synthesis of the acute phase protein response (Rose-John, 2018).

Chronic inflammation becoming more widely recognized as a serious problem due to its role in a number of diseases and conditions, such as diabetes, obesity, cardiovascular, malnutrition, and cancer. Plasma IL-6 has lately been associated with mortality in non-kidney patient categories and cardiovascular morbidity, according to significant epidemiological data (Magno *et al*., 2019). Furthermore, in people with end-session renal illness, a high IL-6 level a powerful indicator of a poor prognosis (ESRD). TNF-α, IL-1b, physical exercise, bacterial endotoxins, and oxidative stress all lead to the release of interleukin-6 from activated monocytes, adipocytes, macrophages, fibroblasts, and endothelial cells (Kamińska *et al*., 2019).

1.5.2 Tumour Necrosis Factor-α

Tumor necrosis factor (TNF-α) is a pro-inflammatory cytokine primarily produced by T-lymphocytes, although other cell types such as vascular endothelial cells, white blood cells, mesangial cells, and renal tubular epithelial cells also contribute to its

production. TNF-α belongs to a family of cytokines that can exist as both cell-bound and soluble forms, and it serves various functions including lymphoid development, inflammation, and apoptosis. Its cytotoxic properties confer protection against tumor formation, and it also plays a role in tissue repair, regeneration, and proliferation. Under normal conditions, TNF-α is present in low concentrations in the circulation. However, its production and secretion increase in the context of inflammation (Mehaffey & Majid, 2017).

Until recently, the biological functions of TNF-α in normal renal function were not well-understood, and it was commonly believed that its activity was mainly important in pathological situations associated with chronic inflammatory disorders. Recent research has shed light on the involvement of TNF-α in controlling normal renal function. For instance, histological features of angiotensin II-dependent hypertension, such as the expansion of vascular smooth muscle cells and the proliferation and infiltration of monocytes in the kidneys, have been found to be linked to increased TNF-α production (Li *et al.*, 2018).

1.5.3 Vitamin-D

In chronic renal disease and other chronic disorders, vitamin D deficiency, defined as serum levels below 30 pg/ml, is prevalent and associated with adverse outcomes. However, research on interventions and randomized controlled trials in this field is still limited. Due to seasonal variations in sun exposure, it is important to regularly evaluate serum vitamin D levels. Nevertheless, nephrologists have long employed various forms of vitamin D in the treatment and prevention of hyperparathyroidism in renal failure.

Selective active vitamin D metabolites such as paricalcitol and maxacalcitol have been used to reduce circulating hyperparathyroidism (PTH) levels, while causing minimal changes in phosphate and calcium concentrations. Determining the ideal dosage and efficacy of co-administering nutritional vitamins with active vitamin D compounds, as well as selecting the appropriate nutritional vitamin D to prescribe, remain subjects of debate. This aspect is crucial for the treatment of chronic renal disease patients, as they often experience vitamin D deficits (Ghasemian *et al*., 2022).

1.6 Potential Biochemical Variables in Chronic Kidney Disease

The factors associated with chronic kidney failure lead to the accumulation of harmful waste products in the body and an increase in fluid and electrolyte imbalances. These imbalances can have significant effects on various organs and systems in the body, including the cardiovascular system, the skeletal system, and the central nervous system. One of the most common complications of chronic kidney failure is cardiovascular disease. This is because the kidneys play a crucial role in regulating blood pressure and fluid balance in the body. When the kidneys fail, blood pressure can rise, and excess fluid can accumulate in the body, which can increase the risk of heart disease and stroke (Cozzolino *et al.*, 2018; Vallianou *et al.*, 2019).

Chronic kidney failure can also lead to bone disease, as the kidneys play a key role in regulating the levels of calcium and phosphorus in the body. When the kidneys fail, these minerals can accumulate in the blood, which can cause bone loss and increase the risk of fractures (KDIGO., 2017). In addition to these complications, chronic kidney failure can also have significant effects on the central nervous system. This is because the

kidneys play a role in regulating the levels of certain neurotransmitters and hormones in the body, including dopamine and parathyroid hormone. When the kidneys fail, these levels become imbalanced, which can lead to cognitive impairment, depression, and other neurological symptoms (Valdivielso *et al.,* 2019).

Dialysis can help to mitigate some of these effects by removing excess fluids and waste products from the body. However, dialysis can also have its own side effects, such as low blood pressure, muscle cramps, and infections. Additionally, the process of dialysis can alter the levels of various substances in the blood, which can affect the accuracy of medical tests and make it more difficult to monitor and manage the health of patients with chronic kidney failure (De Oliveira *et al.,* 2012; Tanaka *et al.,* 2020).

1.7 Problem Statements

The increasing global prevalence of kidney failure, often resulting from lifestyle factors and diseases like diabetes and hypertension, poses a significant public health concern. This study investigates the complex effects of dialysis, a crucial treatment for kidney failure, on blood substance levels, which influences the accuracy of medical tests and complicates patient health management. Specifically, it examines how dialysis sessions impact certain biochemical parameters, including cytokine levels (IL-6, TNF-α) and adipocytokines (Chemerin, Visfatin), which are vital for evaluating and treating end-stage renal disease patients. Additionally, the research explores the relationship between these biomarkers and other potential biochemical parameters, assessing whether haemodialysis can stabilize or improve their levels. Ultimately, this study aims to offer deeper insights into the physiological effects of dialysis and guide more effective

management strategies for patients, while also evaluating the efficacy of haemodialysis in treating end-stage renal failure and its potential risks on other functioning organs, as determined through monitoring test levels.

1.8 Hypothesis

This study hypothesises that levels of tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), vitamin D, serum visfatin, serum chemerin, and other biochemical variables differ significantly between end-stage renal disease (ESRD) patients and healthy individuals. The study also postulates that the dialysis process has a measurable effect on the levels of these biomarkers in ESRD patients. Additionally, there are significant correlations between adipokine, inflammatory marker, and metabolic parameter levels in ESRD patients.

1.9 Objectives of Study

This study aims to assess the effects of dialysis sessions on cytokine levels, adipocytokines, and biochemical parameters in 100 End-Stage Renal Disease (ESRD) patients from the Ramadi district of Iraq, focusing on analyzing the correlations and comparisons between these tests to enhance understanding and help prevent relapse in haemodialysis patients:

1- To determine the levels of TNF-α, IL-6, vitamin D, serum visfatin, serum chemerin and biochemical variables in ESRD patients in comparison to healthy group.
2- To evaluate the effect of dialysis process on the levels of the studied parameters in ESRD patients.
3- To correlate the levels adipokines, inflammatory markers and metabolic parameters in ESRD patients.

1.10 Scope of Study

The scope of this study is to investigate the correlation between the levels of cytokines, specifically TNF-α and IL-6, and Vitamin D in patients undergoing dialysis. The study aims to examine the association of these cytokines with metabolically active adipocytokines, such as Visfatin and Chemerin, as well as various biochemical parameters. The investigation will be conducted before and after three dialysis sessions to assess the effect of multiple dialysis sessions with the dialysis membrane on the aforementioned factors. The study intends to evaluate the impact of dialysis sessions on cytokine levels, adipocytokines, and biochemical parameters, with a focus on

understanding the correlation and comparison between all the tests. The ultimate goal is to gain insights that can help prevent relapse in patients undergoing haemodialysis. The research will involve a cohort of 100 End-Stage Renal Disease (ESRD) patients in the Ramadi district of Iraq.

CHAPTER 2

LITERATURE REVIEW

2.1 Epidemiology of Chronic Kidney Disease

Chronic Kidney Disease (CKD) is a significant and escalating global health challenge. Affecting more than 10% of the global population, this equates to over 800 million individuals worldwide. The disease is more prevalent among older individuals, women, racial minorities, and those with diabetes mellitus and hypertension. CKD poses a particularly heavy burden in low- and middle-income countries, which are often less equipped to manage its consequences (Kovesdy., 2022).

Persistent and systemic low-grade chronic inflammation (CI) is an important part of the poor prognosis in CKD, especially for patients with advanced disease. For example, chronic inflammation worsens anemia and promotes atherosclerosis. Therefore, chronic inflammation deserves our attention in the context of CKD (Yan & Shao, 2023).

As of 2017, the number of patients affected by CKD was estimated at 843.6 million globally, a rise partly attributed to increasing risk factors such as obesity and diabetes mellitus. This increasing trend in CKD cases underscores the urgent need for enhanced prevention and treatment strategies (Jager *et al.*, 2019).

The global prevalence of CKD, according to a comprehensive systematic review and meta-analysis, was found to be 13.4% for CKD stages 1–5 and 10.6% for stages 3–5. The prevalence differs across regions and income levels, with lower prevalence in high-income countries compared to low- and middle-income countries. The age-standardized

global prevalence of CKD stages 3–5 in adults was 4.7% in men and 5.8% in women (Hill *et al.,* 2016).

CKD has become one of the leading causes of death worldwide. According to the Global Burden of Disease (GBD) reports, the all-age mortality rate attributed to CKD increased by 41.5% between 1990 and 2017. CKD was the 19th leading cause of years of life lost in 2013, rising from the 36th in 1990, and is projected to become the fifth highest cause of years of life lost globally by 2040. This rise is more pronounced in certain regions like Latin America, the Caribbean, Southeast Asia, East Asia, Oceania, North Africa, and the Middle East (Abubakar *et al.,* 2015; Bikbov *et al.,* 2020; Foreman *et al.,* 2018). The epidemiology of CKD varies depending on the region and population, but it generally involves a higher risk of mortality and morbidity compared to individuals without CKD. In the Arab world, the prevalence of CKD is increasing due to various factors, such as lifestyle, socioeconomic conditions, and the rising burden of non-communicable diseases (NCDs) (Farag & Al Wakeel., 2011). The regional specificities of Iraq, especially in areas like Al-Anbar, when juxtaposed with broader Middle Eastern trends, provide a nuanced understanding of the prevalence, causes, and challenges in managing renal diseases.

In Northern Iraq, particularly the Kurdish provinces, studies focusing on glomerulonephritis (GN) and ESRD revealed unique patterns. A significant study conducted through renal biopsies in 2012-2013 and analysis of Sulaimaniyah dialysis center records showed that focal segmental glomerulosclerosis (FSGS) was predominant, constituting 35% of GN biopsies. The peak age for GN diagnosis was 35-44 years, with a notable decline in age-specific rates after 45 years. This region's lower ESRD incidence, estimated at 60 per million, is attributed to lower rates of renal disease in the elderly and

infrequency of diabetes, contrasting with higher incidences in Jordan and the US (Ali *et al.,* 2018).

Concurrently, the Al-Anbar region provides a more detailed local perspective. A study at the Al-Ramadi Teaching Hospital's haemodialysis unit from April 2008 to April 2009 assessed 230 haemodialysis patients, revealing a CRF prevalence of 141 patients per million population. Diabetes mellitus and hypertension were the leading causes, found in 33% and 22.6% of patients, respectively. The prevalence of obstructive uropathy (17.3%) was notably higher than in other regions, attributed to the high incidence of renal stone disease and late or incomplete treatment (Awad., 2011).

This regional pattern contrasts with broader Iraqi and Middle Eastern contexts, where the incidence of CKD is rising due to an aging population and higher incidences of diabetes and hypertension. In Al-Anbar, the mean age of CRF patients was slightly lower than in countries like the USA and Saudi Arabia, suggesting regional variations in age distribution of renal diseases (Al-Jiffri *et al.*, 2003; Awad., 2011; Shigidi *et al.,* 2009).

In terms of glomerular diseases as a cause of ESRD, the study in Al-Anbar found a lower incidence (4.3%) compared to places like Aleppo-Syria and Egypt. This disparity could be due to underdiagnosis or misclassification of chronic glomerular diseases, given the limitations in diagnostic practices and the lack of uniform biopsy procedures across Iraq (Awad., 2011; Moukeh *et al.,* 2009).

The findings from Al-Anbar, when compared with regional data, underscore the significant impact of diabetes and hypertension as etiologies of renal diseases. This

indicates a potential for prevention through better management of these conditions. However, the study also pointed out the need for improvements in the healthcare system, including early diagnosis and referral, to reduce the morbidity associated with renal diseases (Awad., 2011).

In conclusion, the study of renal diseases in Iraq, both in specific regions like Al-Anbar and the broader Kurdish areas, provides valuable insights into the complexities of renal epidemiology in the Middle East. These findings highlight the necessity of localized healthcare strategies and the importance of addressing systemic challenges in healthcare delivery. The variations in disease patterns, age distribution, and etiology emphasize the need for tailored approaches to healthcare and epidemiological research in diverse regional contexts.

2.2 Renal System

Renal system, also known as the urinary system, consists of the kidneys, bladder, urethra, and ureters. Nephrons are millions of functional units that make up each kidney. The functions of the renal system include waste elimination, regulation of blood pressure and blood volume, control of metabolites and electrolytes, and regulation of blood pH (Breshears & Confer, 2017).

The kidneys are the most important organs in maintaining homeostasis as they regulate the blood's acid-base balance and water-salt balance. The renal arteries supply a large amount of blood to the kidneys, which leaves the kidneys through the renal vein. Waste in the form of urine exits the kidneys through the ureters, which are muscular tubes

that transport urine to the bladder, where it is stored and then released from the body through urination (Srivastava, 2018).

Urine is a liquid excreted through the urethra, produced by the kidneys, collected in the bladder, and expelled from the body. Urine is used to eliminate excess minerals and vitamins from the body, as well as waste products from the blood. The female and male urinary systems are very similar, differing only in the length of the urethra (Abelson *et al.*, 2018).

2.2.1 Functions of The Renal System

The renal system has various roles, including the removal or excretion of waste products from the body, such as creatinine, urea, and uric acid. It also plays a role in electrolyte balancing, regulating substances like sodium, calcium, and potassium. The urinary system is involved in maintaining acid-base homeostasis, controlling blood pressure, and regulating blood volume (Gormley-Fleming, 2021).

Excretion is the vital function of the urinary system, which involves the elimination of waste products of metabolism and other non-essential components from the body. The urinary system also regulates the amount of water excreted in urine to maintain proper fluid volume (Srivastava, 2018). Therefore, the system is responsible for maintaining the concentrations of various electrolytes in body fluids and ensuring the normal pH of the blood. The primary function of the kidneys is to maintain a stable internal environment (homeostasis) to support optimal cell and tissue metabolism. This is achieved through the filtration of the blood for urea, toxins, mineral salts, and other waste substances. The kidneys play a vital role in regulating the balance of salt, water, and

electrolytes in the body. It is essential for at least one kidney to function properly for life to be sustained (Huff, 2020).

2.2.2 Anatomy and Physiology of The Kidney

The kidneys are a pair of retroperitoneal organs, bean-shaped, reddish-brown in colour and are located in the posterior part of the abdomen on each side of the vertebral column. Each kidney is approximately the size of a fist, weighing about 150 grams and measuring 11cm x 6cm. The functional unit of the kidney consists of millions of tiny filters called nephrons, which are present in each kidney. The renal artery enters the kidney through a depression on its concave side, while the renal vein and ureter exit the kidney.

The kidney has three primary regions: the renal cortex, renal medulla, and renal pelvis. The renal cortex is the outer granular layer, while the renal medulla is the inner layer that appears radially striated. The renal pelvis is located at the center of the kidney and is continuous with the ureters (Gormley-Fleming, 2021; Neuendorf, 2020). A visual representation of the renal anatomy and physiology can be seen in Figure 2.1.

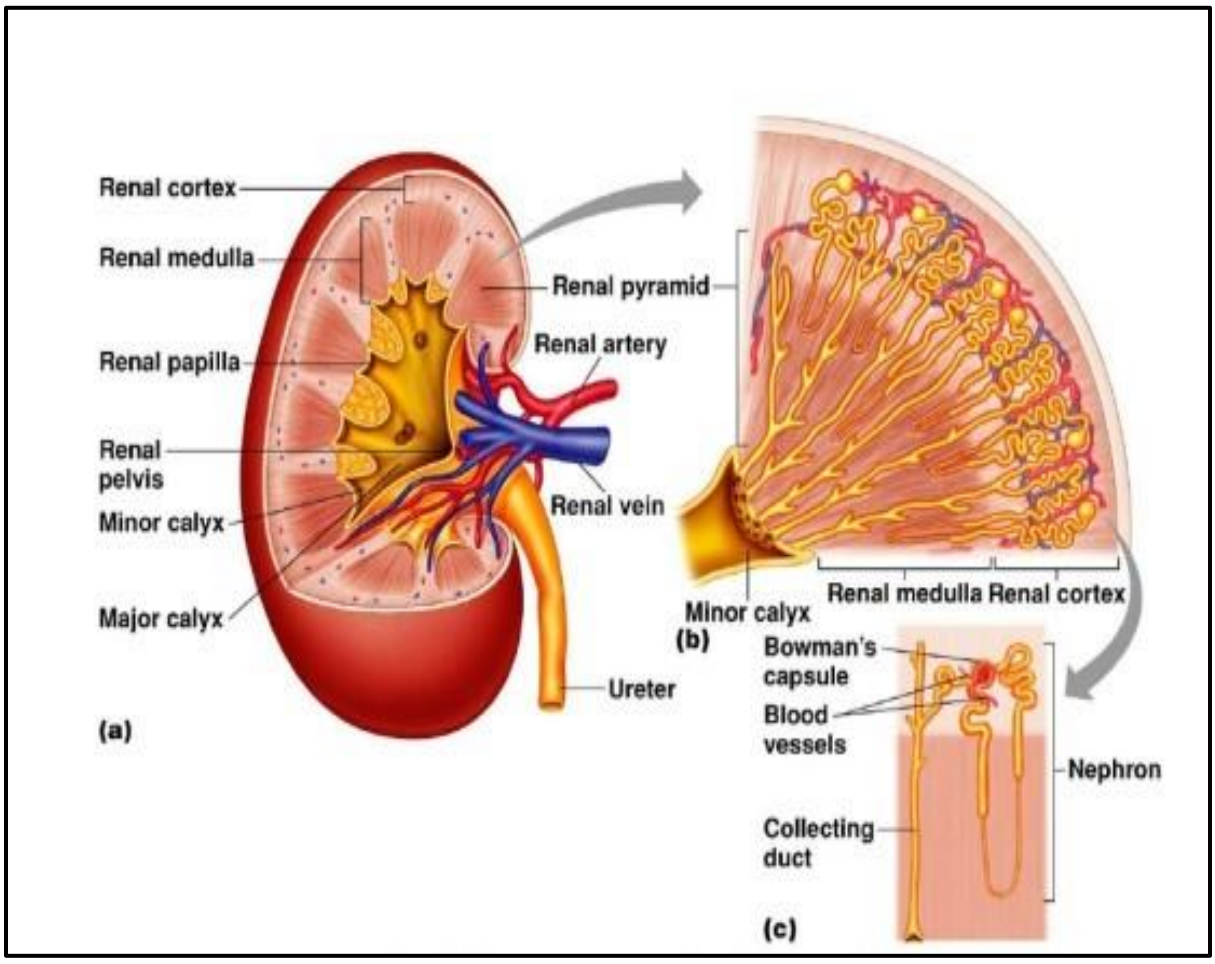

Figure 2.1 Anatomy and Physiology of The Kidneys. (a) It Presents a Cross-Section of The Kidney, Showing The Outer Cortex, Inner Medulla with Striated Pyramids, the central Pelvis leading to the Ureter, and the Renal Artery and Vein. (b) It offers an expanded view of the Renal Medulla, focusing on the Renal Pyramids and the Blood Filtration and Urine Collection process. (c) It provides a detailed view of a Nephron, highlighting Blood Filtration through the Bowman's Capsule and the Urine's journey through the Collecting Duct to the Renal Pelvis. Obtained from (Koeppen & Stanton, 2018).

The kidneys play crucial roles and perform essential functions within the human body. One of their primary functions is the regulation and maintenance of various aspects of plasma composition, including ionic composition, osmolarity, volume, and hydrogen ion concentration (pH). Additionally, the kidneys are responsible for the elimination of metabolic waste products and extraneous substances present in the plasma. Furthermore, it is worth noting that the kidneys also secrete hormones, such a hormone is erythropoietin (EPO) (Abdelrasoul *et al*., 2021).

2.2.3 Renal Function

In nephrology, assessing renal function provides valuable insights into the health status of the kidneys and their vital role in maintaining the body's internal balance through various physiological processes. One key measure of renal function is the glomerular filtration rate (GFR), which represents the amount of fluid filtered by the kidneys within a given period. Another measure is the creatinine clearance rate (Ccr), which indicates the volume of blood plasma cleared of creatinine per unit of time. Ccr is a valuable approximation of GFR and can be determined by comparing levels of certain substances in the blood and urine, or by using formulas that utilize blood test results (Fenton *et al.*, 2018). The results of GFR and Ccr tests, or their estimated values, provide valuable insights into the kidneys' excretory capacity. Additionally, albuminuria, GFR categories, and the underlying etiology of renal disease are assessed to evaluate the presence and severity of kidney disease. These factors collectively aid in the classification and staging of kidney disease (Shahbaz & Gupta, 2019).

2.2.4 Methods of Estimation of GFR

The normal range of glomerular filtration rate (GFR), adjusted for body surface area, is similar in both men and women. In young adults, the normal GFR is greater than 90 ml/min/1.73m². In children, GFR, as measured by inulin clearance, remains close to about 110 ml/min/1.73m² until around the age of 2 in both sexes, after which it gradually decreases. After the age of 40, GFR progressively decreases with age by approximately 0.4-1.2 ml/min per year (Fenton *et al.,* 2018).

Estimation of GFR is crucial for assessing renal function and can be achieved using various methods. These include clearance tests such as inulin clearance and creatinine clearance, as well as equations like the Modification of Diet in Renal Disease (MDRD) formula and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula (Rivera *et al.,* 2018). Clearance tests are based on the principle that the rate of elimination of a substance from the body by the kidneys (through urine) reflects the rate at which it is filtered. Inulin clearance is a direct method, where inulin, a polysaccharide not produced by the body, is administered intravenously to measure GFR. Creatinine clearance, on the other hand, uses creatinine, a byproduct of muscle metabolism, to estimate GFR. However, it is not a perfect indicator due to slight secretion of creatinine by the tubules (Breshears & Confer, 2017; Shahbaz & Gupta, 2019).

Both MDRD and CKD-EPI formulas use serum creatinine, age, and other parameters like gender and ethnicity to estimate GFR. While MDRD has been traditionally used, CKD-EPI has shown improved accuracy and reduced bias, especially at higher GFRs (Palacio-Lacambra *et al.,* 2018; Tang *et al.,* 2022).

By strea mlining the measurement methods of GFR, this is a concise overview of the various approaches used in clinical practice to assess kidney function.

2.3 Diseases of Kidney

Nephropathy, also known as kidney disease or renal disease, is a condition in which the kidneys are damaged or diseased. Inflammatory kidney disease is known as nephritis. Non-inflammatory nephropathy is referred to as nephrosis. Kidney disease typically results in kidney failure, (renal failure) to varying degrees, depending on the kind of disease. Disease signifies the structural and etiologic disease entity, while failure indicates the dysfunction (lack of working well, i.e., impaired renal function). However, these meanings overlap in common usage, for example, the terms chronic kidney disease and chronic renal failure are frequently used interchangeably. Acute kidney disease was previously known as acute renal failure, but nephrologists now refer to it as acute kidney damage (Rajasekaran *et al*. 2021).

2.3.1 Diabetic Nephropathy

Diabetic nephropathy, also called as (Kimmelstiel-Wilson) syndrome as well as intercapillary glomerulonephritis, is a chronic kidney disease characterized by capillary angiopathy in the glomeruli of the kidney. It is characterized by nephrotic syndrome and glomerulosclerosis scarring. It is linked to poorly managed diabetes mellitus (DM) and is the leading cause of dialysis in many affluent nations (Akhtar *et al*., 2020).

Although the exact origin of diabetic nephropathy is unknown, elevated blood sugar levels in diabetic individuals, the creation of advanced glycation end products, and cytokines are thought to play a role. Diagnosis of diabetic nephropathy is usually based