An-Najah National University

Faculty of Graduate Studies

THE CORRELATION BETWEEN INTERLEUKIN-6 AND BONE MINERAL DENSITY AMONG HEMODIALYSIS PATIENTS IN PALESTINE

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Dedication

This thesis is dedicated to:

Allah, the great, my source of strength and inspiration.

My mom for her endless, causeless love and support.

My dad, whose encouragement has been my driving force, brought me up to be where I am today.

My lovely sisters understand me, encourage me, and are my confidants, and your endless support means the world to me.

My lovely friends who have stood with me from childhood until now.

For everyone who has tough me anything along the way, and who easy way of science for every knowledge seeker.

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Declaration

I, the undersigned, declare that I submitted the thesis entitled:

THE CORRELATION BETWEEN INTERLEUKIN-6 AND BONE MINERAL DENSITY AMONG HEMODIALYSIS PATIENTS IN PALESTINE

I declare that the work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

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THE CORRELATION BETWEEN INTERLEUKIN-6 AND BONE MINERAL DENSITY AMONG HEMODIALYSIS PATIENTS IN PALESTINE

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Abstract

Background: Chronic kidney disease (CKD) is a significant public health challenge worldwide. CKD have systemic effects on the body, including cardiovascular diseases (CVDs), hematologic problems, metabolic abnormalities, and bone diseases, which are then referred to as chronic kidney disease-mineral and bone disorders (CKD-MBD). Therefore, monitoring bone density in chronic kidney disease patients is very crucial. This study aims to identify the association between IL-6 and clinical parameters with Bone density in chronic kidney disease patients, to find out the association between IL-6 and the severity of bone mineral disease on (Dual-energy X-ray absorptiometry) DEXA scan to measure IL-6 as a new marker for bone remodelling and reabsorption in renal osteodystrophy patients as determined by bone mineral density (BMD).

Methods: A cross-sectional study was conducted among CKD patients in An-Najah National University Hospital, Nablus, Palestine dialysis unit. 138 samples were collected from the patients between April 2022 and May 2022. Interleukin-6 cytokine was tested using the manual ELISA method. A dual-energy X-ray absorptiometry (DEXA) scan was used to evaluate BMD, SPSS were used to analyze data.

Results: The results indicate that most patients included in the study had osteoporosis with a prevalence of 60%, 53%, 47% and 50%, 44.7, and 33.8 for osteopenia at the spine, hip, and neck, respectively.

IL-6 is normal within our participants, and there is no relationship between IL-6 and Dexa scan. On the other hand, this study revealed that there is a correlation between IL-6 and albumin (p value< 0.001), ferritin (p-value = 0.011), iron (p-value = 0.008), transferrin saturation (p-value = 0.013), and hemoglobin concentration (p-value = 0.018).

Patients who had more CVDs significantly had higher IL-6 than patients who had not. In addition, the IL-6 value for patients with only CVDs is higher than that for those with DM or both CVD and DM and higher than that for those with none of them.

Conclusion: The main promising finding is that Palestinian CKD patients have low bone density and elevated levels of IL-6, which were linked with low albumin, iron, transferrin saturation, and hemoglobin but increased ferritin concentration. It also demonstrated that CVDs could be related to high IL-6 concentration in those patients.

Keywords: Chronic kidney disease; IL-6; renal osteodystrophy; CVDs; BMD.

Chapter One

Introduction

1.1 Background

Research on Chronic Kidney Disease (CKD) has a long tradition. CKD has been considered a worldwide public health challenge (1). CKD have systemic effects on the body, including cardiovascular diseases (CVDs) (2), hematologic problems (3), metabolic abnormalities, and bone diseases, which are then referred to as chronic kidney disease-mineral and bone disorder CKD-MBD (4).

The prevalence of CKD in developing is higher than in developed countries (5). The incidence of CKD patients is increasing globally (6). In Palestine, there are few studies on CKD patients. However, in 2017, 1216 patients were on hemodialysis in west bank, and the number is increasing (6).

Therefore, monitoring bone density in chronic kidney disease patients is very crucial. This study aims to identify the association between IL-6 and clinical parameters with bone density in chronic kidney disease patients.

1.2 Renal system: definition and function

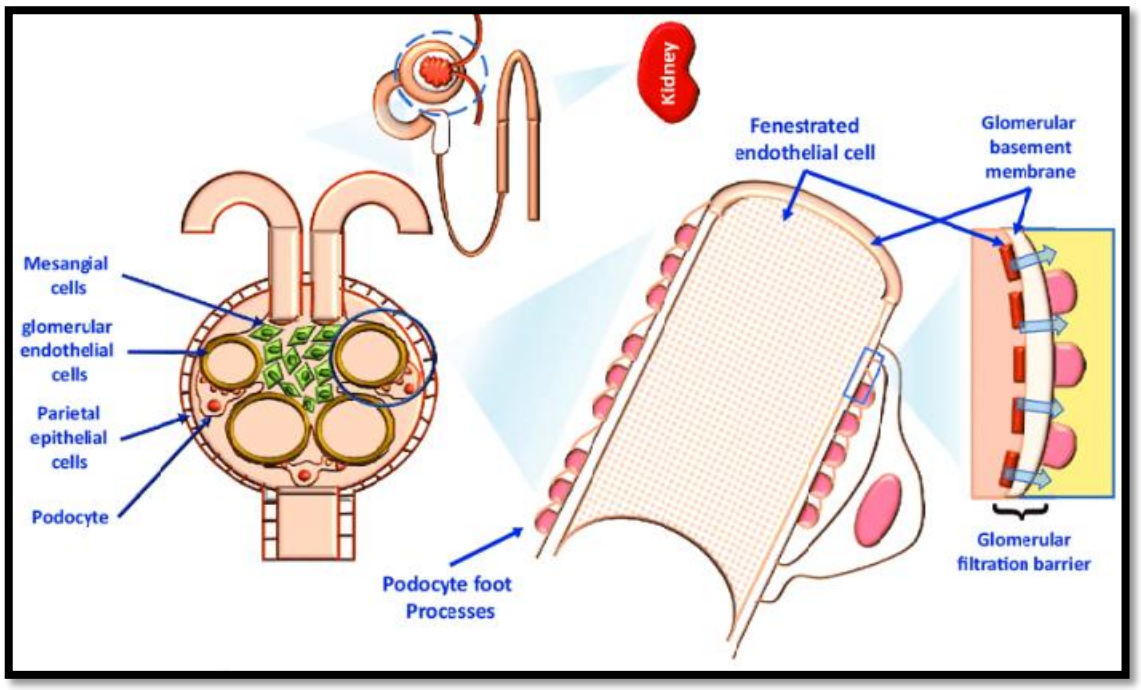
The renal system comprises the kidneys, ureters, and urethra. Collectively, this system filters approximately 200 liters of fluid daily from the blood flowing through the kidneys. Previous work demonstrates that this process facilitates the elimination of toxins, metabolic waste products, and excess ions, all while preserving the essential components of the blood (7). The kidneys regulate plasma osmolality by controlling the concentration of water, solutes, and electrolytes in the blood, thereby maintaining long-term acid-base balance. They produce erythropoietin, stimulating the synthesis of red blood cells. Moreover, the kidneys generate renin to manage blood pressure and convert vitamin D into its active form, known as calcitriol (7).

Glomerular filtration is a passive process wherein fluid and solute inside the blood are forced through a barrier membrane by hydrostatic pressure without energy. The filtration barrier membrane is made up of three layers. The first layer is the fenestrated endothelium of glomerular capillaries, which allows all blood substances to pass through other than cells. The second layer is the basement membrane, a negatively

charged physical barrier that prevents proteins from permeating. The last layer is the glomerular capsule, which has a podocyte that creates more precise filtration. (See figure 1) Hydrostatic pressure, the primary filtration force which drives blood along capillaries, determines the volume of blood that crosses the filtration membrane (7).

Figure 1

Structure and components of the renal glomerular filtration system (8)



Glomerular filtration rate (GFR), or the fluid volume filtered in a minute, is determined by the net filtration pressure, the total amount of surface area that is accessible for filtration, and the permeability of the filtration membrane (net filtration pressure). The GFR ranges from 120 to 125 ml/min. It is internally and extrinsically regulated to sustain the GFR in the normal range (7).

1.3 Calcium and phosphate regulation

Calcium and phosphate hemostasis are necessary for bone mineralization and growth, regulated mainly by the kidney (6), which reabsorbs between 98 and 99 per cent of the calcium filtered out through renal tubules. This hemostasis depends on several interactions between body systems and regulatory hormones, including Parathyroid hormone (PTH) (9).

PTH impacts bone, kidney, and gastrointestinal function and can regulate calcium and phosphate levels. Therefore, any alteration in this ion concentration will influence the target organs' functionality, particularly bone, and affect these hormones (9).

Calcium regulation depends on many biological, therapeutic, pathologic, and hormonal factors. PTH promotes calcium absorption and is the most crucial regulator of the hormone. It is a polypeptide released by the parathyroid gland when the blood calcium level decreases. Thus, maintaining calcium homeostasis is the primary physiological function of the parathyroid gland through the calcium-sensing receptor (CaSR), a receptor embedded in the parathyroid cells' cell membrane that recognizes changes in serum calcium levels, resulting in changes in PTH secretion (10).

It is well acknowledged that phosphate is an essential electrolyte in the human body, and it’s most abundant amount of phosphorus reserves is found in bone. The maintenance of serum phosphate (Pi) levels within (2.5-4.5 mg/dl in adulthood) is essential for many key cellular processes, such as metabolic activities, bone formation, cell signaling, or as a component of phospholipids and nucleic acids (10).

To keep serum phosphate levels within the normal range, a complex interaction between phosphate absorption in the gut, exchange with bone reserves, changes between intracellular and intravascular compartments, and renal excretion must occur (10), (11), (12).

1.4 Fibroblast growth factor-23 (FGF-23) in renal system

FGF-23 is a protein secreted from osteocytes and osteoblasts. It is considered one of the critical regulators of phosphate and calcitriol levels. It is produced in response to high phosphate, calcium and PTH serum levels (13). FGF-23 can work by inhibiting the sodium-phosphate co-transporter in the proximal convoluted tubule, stimulating phosphate excretion through the urine. FGF-23 can also suppress 1α-Hydroxylase and induce 24-hydroxylase in proximal renal tubules, lowering calcitriol synthesis and resulting in 25-hydroxyvitamin D insufficiency (14).

1.5 Vitamin D production and its metabolism

Cholecalciferol, or vitamin D3, is a fat-soluble vitamin found in food and can be produced by the skin from 7-dehydrocholesterol when exposed to ultraviolet light(15).

Hepatic enzyme 25-hydroxylase prompts the hydroxylation of vitamin D3 to form 25-hydroxyvitamin D, known as calcidiol. 25-hydroxyvitamin D can leave the liver in the bloodstream as an inactive metabolite. This inactive metabolite is considered the precursor form of active vitamin D, and the best predictor of the overall storage of vitamin D is the plasma concentration of 25(OH)D (10).

25-hydroxyvitamin D is further hydroxylated in the kidney by the 1a-hydroxylase enzyme to 1,25(OH)2D, or 24,25-dihydroxy vitamin D, the most active form of vitamin D. This active form of vitamin D (1, 25(OH)2D) gets into the blood and travels to the small intestine, where it regulates the absorption of intestinal calcium(15).

The 1-hydroxylase enzyme found in proximal tubular cells of the nephrons is activated by both hypocalcemia and PTH, promoting the production of 1, 25(OH) 2D. It supports intestinal absorption of calcium and phosphate. In the distal convoluted tubule, 1, 25(OH)2D promotes calcium reabsorption. Additionally, it improves the gastrointestinal tract's ability to absorb calcium from food. All these methods help restore normal serum calcium levels (10), (16).

1.6 Chronic kidney disease: definition, function, classification and consequences

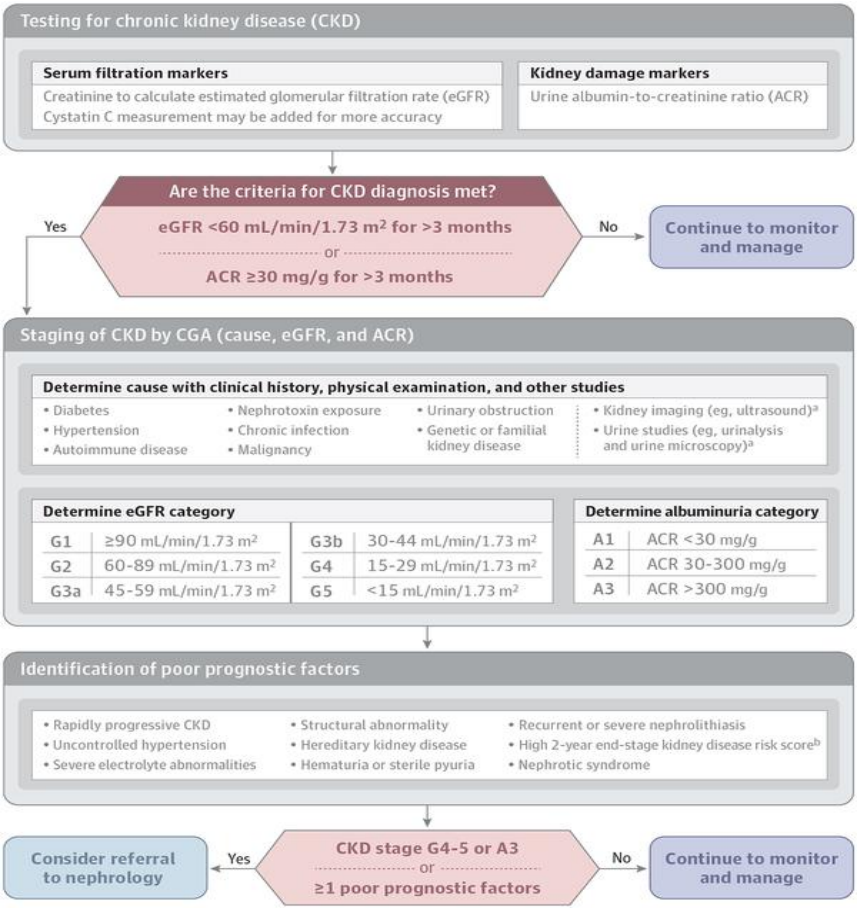
CKD is a significant public health challenge worldwide(1). According to the Kidney Disease Improving Global Outcomes (KDIGO) foundation guideline, CKD is divided to 5 stages, and it is defined as the presence of kidney damage which appears with proteinuria or decreased kidney function, which is shown by decreased GFR of less than 60 ml/min per 1.73 m^2 for three months or more (17). In the case of unclear duration of kidney disorder, physician must differentiate between CKD and Acute kidney injury (AKI) (5).

Some patients may suffer from symptoms such as decreased or absence of urine output, edema, foamy urine, fatigue, hematuria, vomiting, nausea and weight loss (5).

CKD Stage 5, or ESRD, also called renal failure, is characterized by a GFR of less than 15 ml per 1.73 m2 /min (17). It is also considered as the leading cause for mortality and death (18).(See figure 2)

Figure 2

Classification and staging of CKD (4)



The leading causes of CKD in most countries are diabetes and hypertension. According to social health factors and nationality, CKD incidence, prevalence, and progression differ between nations, presumably due to the influence of epigenetics (19). Understanding the leading cause of CKD could help in prognosis and treatment(5).

Systemic inflammation associated with CKD state plays a crucial role in disease progression in CKD patients. Several factors may contribute to the inflammation state, including dialysis machine contamination, reduced cytokines renal clearance, chronic and recurrent infection, buildup of glycosylation end-product, decreased plasma antioxidant and uremia(20), which is a buildup of metabolic end product in blood as a result of damaged kidney function. These factors are also responsible for CKD

consequences, such as cardiovascular dysfunction, hypertension and metabolic acidosis, insulin resistance, anemia, erythropoietin resistance, and endothelial dysfunction (21).

Inflammation in CKD is associated with increased production of inflammation markers and the disease's severity, such as cytokines, acute phase reactant, tumor necrosis factor-α, adhesion molecule and IL-6(21). However, the severity of inflammation depends on the hemostasis between pro-inflammatory and anti-inflammatory cytokines (20).

Moreover, CKD also has systemic effects on the body, including metabolic abnormalities and bone diseases, referred to as CKD-MBD (4). According to KDIGO, CKD–MBD is a systemic disease of bone and mineral metabolism due to CKD. It is characterized by one or more of the following:

Abnormalities are found in the metabolism of vitamin D, calcium, phosphorus, FGF-23 or PTH (22).

Variations also can be found in the volume, linear growth, mineralization, strength, or turnover of bone calcification of soft tissues, such as arteries (22).

Vascular calcification is a characteristic feature of CKD-MBD. It involves the abnormal accumulation of calcium-phosphate salts in vascular structures such as blood vessels, valves, and the heart. It is commonly associated with ageing, diabetes mellitus, CKD, calcific aortic valve disease, various genetic conditions (23), dyslipidemia, high phosphorus and calcium levels, vitamin D deficiency, calcium phosphate binders, and secondary hyperparathyroidism (24).

Vascular calcification serves as a strong predictor of cardiovascular morbidity and mortality within the CKD patient. High serum phosphate levels, which typically appear later in CKD, have been demonstrated to expedite mineral deposition in both vascular walls and heart valves (23). Additionally, FGF-23 is a significant factor in CKD-MBD, which may be participating in the pathogenesis of uremic vascular calcification(23).

Usually, ESRD patients undergo renal replacement therapy either by dialysis or kidney transplantation (25). Hemodialysis is a blood-cleaning treatment involving the use of dialysis machine and a specific filter known as an artificial kidney or dialyzer to filter out a patient's blood to improve azotemia, fluid, electrolyte, and acid-base abnormalities. Hemodialysis is most commonly used to treat acute and chronic kidney

failure (26). The prevalence of ESRD among Palestinians who are on dialysis was 240.3 per million population in 2010, which is similar to neighboring countries, and the number is increasing (27).

1.7 Renal osteodystrophy: definition and pathophysiology

Renal osteodystrophy is identified by deranged bone structure and morphology in individuals with CKD. It is now recognized as a part of CKD-MBD, according to KDIGO (14). Renal osteodystrophy abnormalities include electrolyte and endocrine changes such as calcium, PTH, vitamin D serum levels and phosphate levels which could be high due to phosphorous excretion from the kidney and results in hyperphosphatemia, in addition to the stimulation of FGF-23 release from osteocytes, elevated FGF-23 can raise phosphate excretion, lower phosphate serum level(14). Along with the effect of this change on decreased bone turnover and skeletal mineralization. According to statistics, patients with a GFR<60 mL/min/1.73 m2 are more likely to experience these defects. Renal osteodystrophy happens due to secondary hyperparathyroidism, in which the parathyroid gland makes too much PTH due to calcium loss from another disorder (28).

These events can directly affect the activity of the 1a-hydroxylase enzyme that subsequently decreases 1,25(OH)2D, decreasing calcium and phosphorous absorption from the intestine (28). In addition, calcium starts to decline when GFR reaches 20 mL/min/1.73 m2 (29). CaSR embedded in the Parathyroid gland can sense the decrease in PTH, which can result in increased PTH level, known as secondary hyperparathyroidism. Increased PTH levels allow for compensations through increased phosphorus excretion from the kidneys to the urine and calcium absorption from the gut through vitamin D (28).

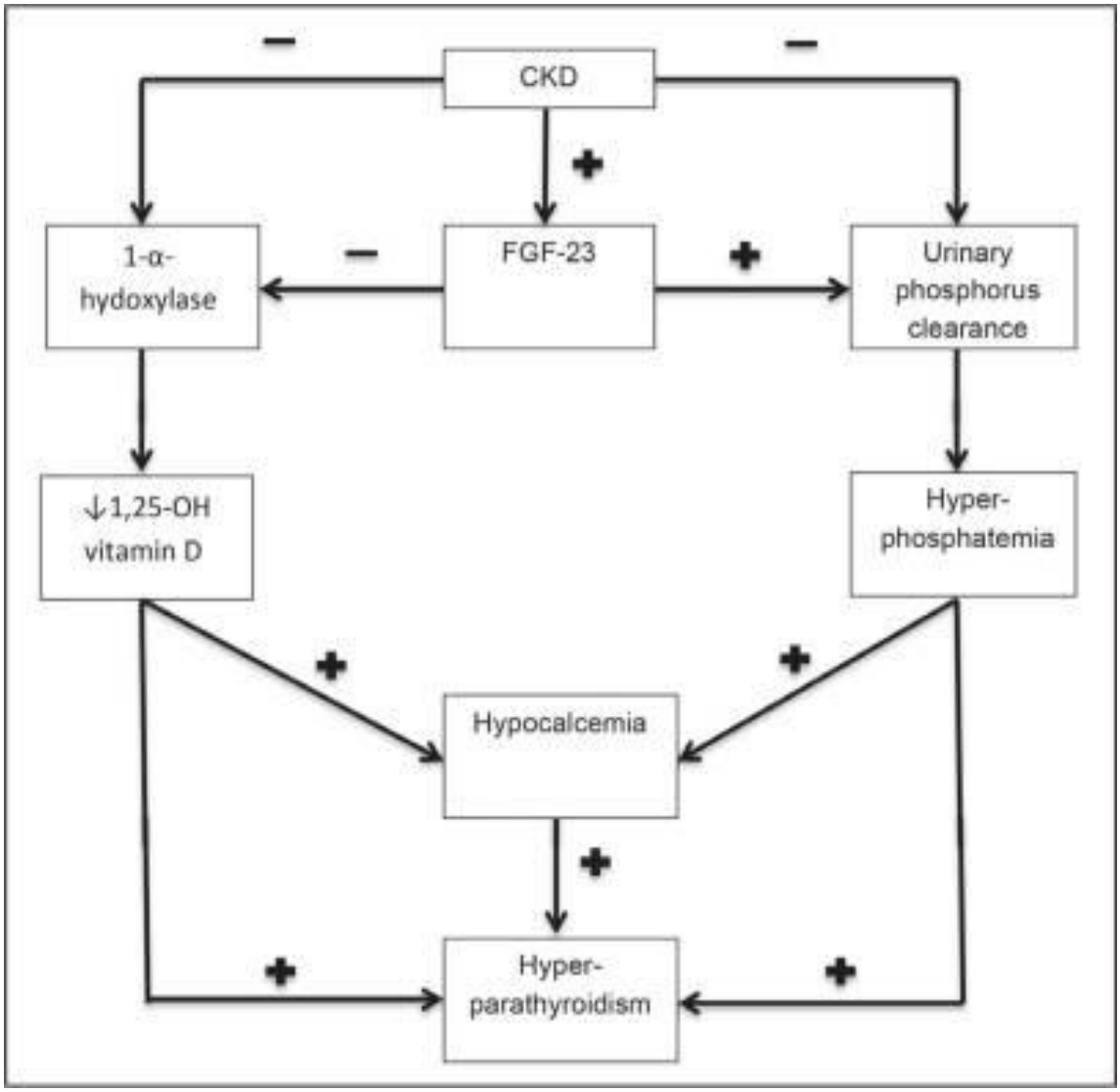
However, when GFR keeps dropping (typically when the GFR falls below 40 mL/min/1.73 m2), these protective compensatory mechanisms stop working, resulting in hypophosphatemia despite high PTH and FGF-23. Furthermore, high phosphate levels bind bioavailability with calcium to form dicalcium phosphate, lowering calcium levels and stimulating the parathyroid gland (14). See figure 3 (14). In response to this continuous stimulation in patients with severe CKD, parathyroid glands develop hypertrophy and hyperplastic changes. Moreover, continuous stimulation of FGF-23 can

result in undesirable effects involving CKD progression, left ventricular hypertrophy, and premature mortality (30).

With this continuous stimulation of PTH and FGF-23, osteoblast function and osteocyte activation may be affected, resulting in high bone turnover, fracture risk, and renal osteodystrophy (13). Thus, as with PTH, FGF-23 can be identified as one of the most important indicators of CKD-MBD (8), which may be linked to a high mortality rate from CVDs in patients with CKD (14).

Figure 3

Pathogenesis of hyperparathyroidism (10)



1.8 Bone marrow

Bone marrow is a spongy tissue located within bones, housing immature cells. It comprises two main types of cells: mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs). Additionally, it contains various other cell types, such as adipocytes, chondrocytes, endothelial cells, immune cells, and fibroblasts (31). MSCs can transform into osteoprogenitor cells, ultimately maturing into osteoblasts, the cells responsible for bone refill or formation and remodeling. On the other hand, osteoclasts, which are involved in bone resorption, originate from HSCs (32).

Osteoblast and osteoclast interact directly to maintain bone hemostasis; any imbalance between these cells in absorption and resorption can lead to bone loss, such as osteoporosis. Osteoclasts work through bone resorption, producing some cavities or holes in the bone, and the cavity produced by this process is refilled by bone formation done by osteoblast. Osteoblast and osteoclast interact with each other by producing molecules and factors like receptor activator of nuclear factor kappa-B ligand (RANKL), receptor activator of nuclear factor kappa-B (RANK) and osteoprotegerin (OPG) (33).

RANKL is a molecule found in normal physiologic conditions on osteoblast cells and is essential for osteoclast stimulation. Rank is the receptor for RANKL found on the surface of progenitor osteoclast and OPG, also known as osteoclastogenesis inhibitory factor (OCIF) (31).

The cooperation of the RANKL/RANK signaling system with its inhibitor, OPG, regulates the activation, survival, formation and differentiation of multinucleated osteoclasts from their progenitors during bone remodeling (31). This cooperation can be done through the direct interaction between RANK and RANKL, which can form osteoclasts. Also, Osteoclast can be activated when it travels to the bone surface, fuses and adheres to the bone surface. This active osteoclast can form an enclosing area rich in actin. It becomes acidified to ensure destroying bone collagen and components of bone through a proteolytic enzyme (34).

The immune system and bone health are closely related. For example, inflammatory cytokines like interleukins are essential for maintaining skeletal homeostasis, and their

dysregulation can disrupt bone homeostasis and remodeling by affecting the ratio of RANKL to OPGs, which can affect osteoclast differentiation (31).

1.9 Interleukin 6 (IL-6): definition, function

Leukocytes are the first known cells to express the cytokine family known as interleukins. Later, it was found that many other cells could produce interleukins. Interleukins are crucial for immune cell activation, differentiation, proliferation and migration; they also exhibit pro-and anti-inflammatory properties. Thus, interleukins can be vital to immune response (35). Receptor complexes that bind with different interleukins have led to their classification into families, the most significant of which are the IL-1 superfamily, IL-6 family, IL-10 family, IL-12 family, and IL-17 family (36).

In 1973, IL-6 was established to be a T-cell-secreted protein that controlled B-cell synthesis of antibodies. Since that day, it has been demonstrated to have a broad spectrum of effects in various cell types, including bone ones, making it a proinflammatory cytokine (37). It is considered a pleiotropic cytokine, which has pro- and anti-inflammatory effects in infections (38).

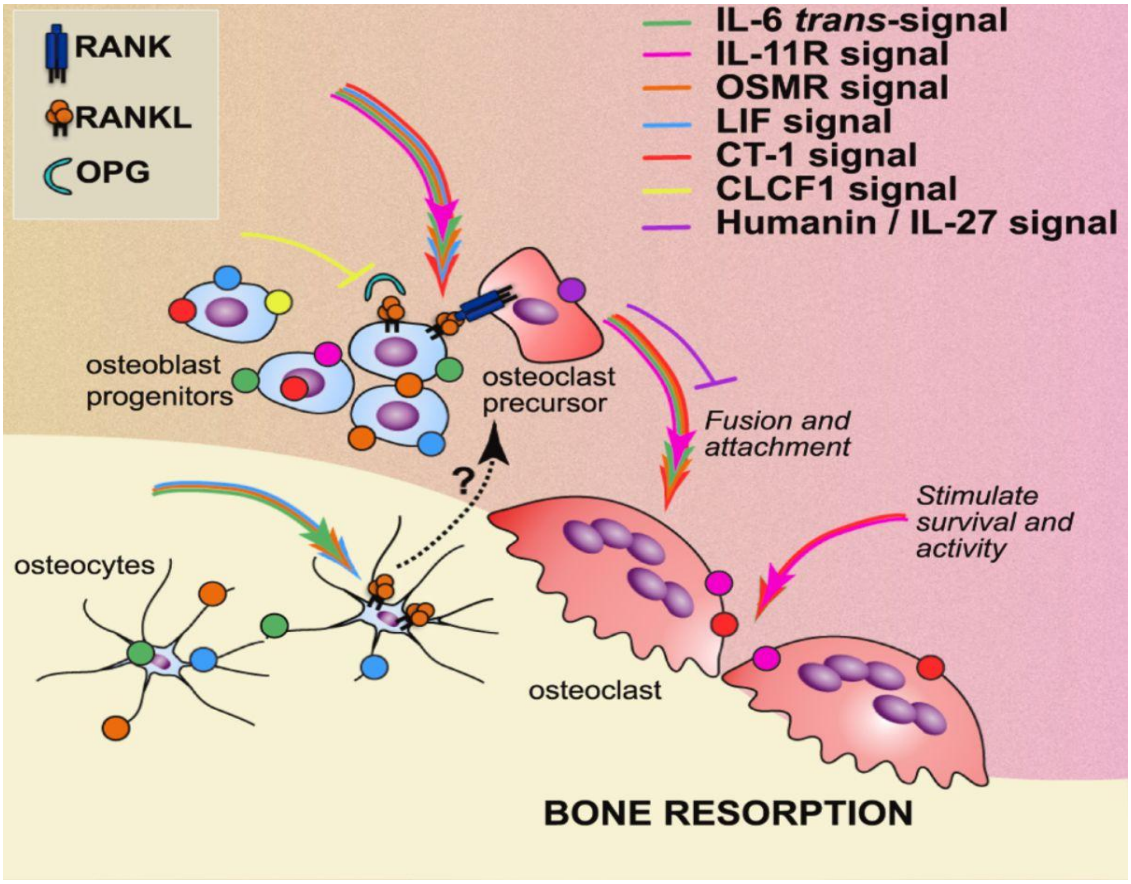
IL-6 exhibits diverse physiological and biological effects, including stimulating IgG, IgM, and IgA production, facilitating T helper cell differentiation, and induction of acute phase reactants from liver cells (39). This broad spectrum of actions highlights the crucial role of IL-6 in the immune response, particularly in scenarios involving lymphocyte activation and recurring or persistent viral, microbial, parasitic, or fungal infections. The elevation of circulating IL-6 levels during infections underscores its significant contribution to the protective functions of the immune system (40).

Moreover, it regulates endothelial function, neuronal function, lipid balance, insulin sensitivity, activity levels, and various metabolic aspects. This is why nearly all types of cells produce IL-6 (40). Typically, IL-6 is either undetectable in the bloodstream or detectable only at low levels (41). In contemporary times, owing to its numerous physiological effects, it is considered a cytokine with hormone-like characteristics (hormone-like cytokine) (40).

IL-6 is also produced by most bone cells, including osteoblasts and osteoclasts, and osteoclasts express the Interleukin-6 receptor (IL-6R) on their surface. Despite that, IL-6 cannot act directly on osteoclasts. Instead, osteoclast can be stimulated and differentiated indirectly by osteoblast through the stimulation of IL-6 to RANKL on the surface of osteoblast (34), which can activate the RANKL pathway, and stimulate osteolysis, inhibit OPG, which inhibits osteolysis, causing bone resorption and osteoclastogenesis through IL-6 trans-signaling (31) (see Figure 4).

Figure 4

Osteoclast activation though RANKL (34)



1.10 IL-6 pathways

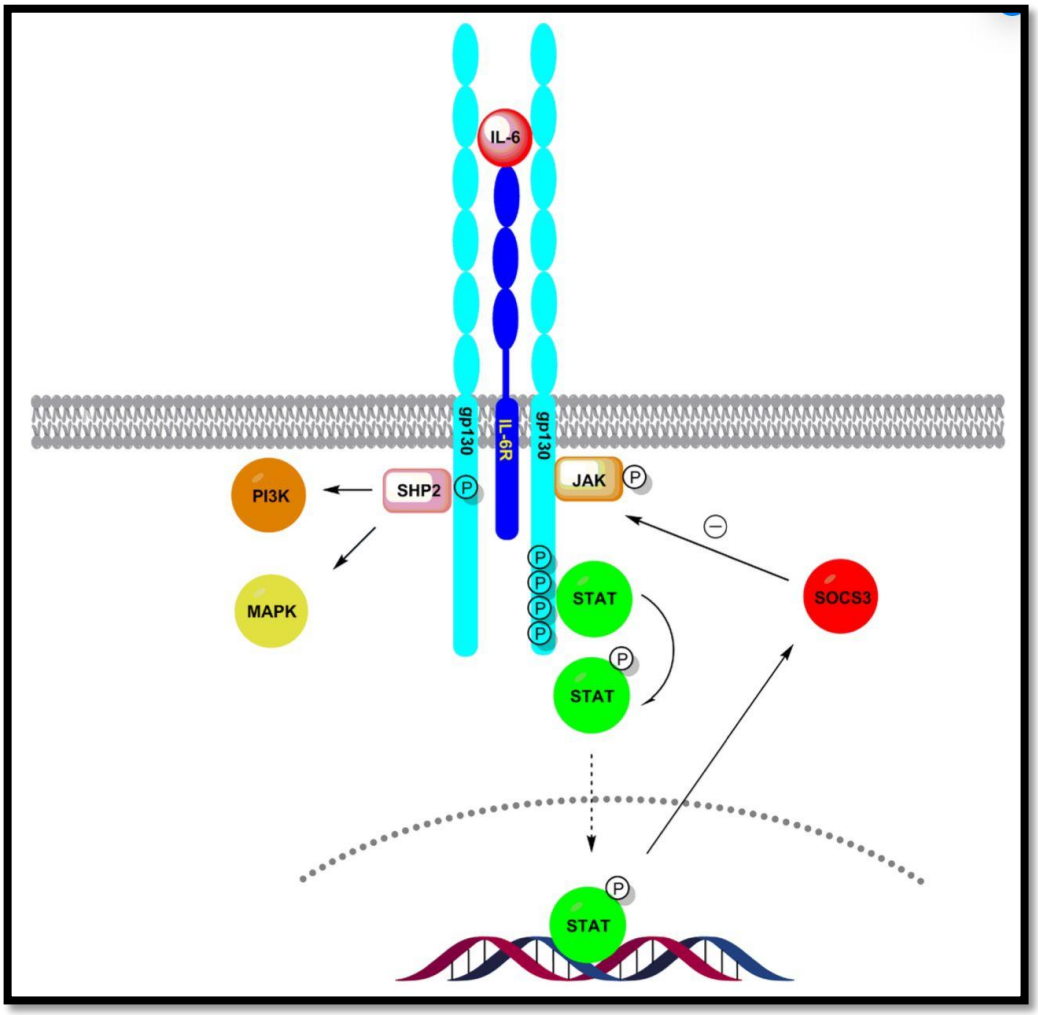
Various mechanisms, such as Adipokines, prostaglandins, Toll-like receptors and other cytokines, control IL-6 expression. However, tumor necrosis factor and IL-1 are two primary basic pro-inflammatory cytokines that induce IL-6 synthesis (40).

IL-6 cytokines can form a signal complex with the co-receptor glycoprotein 130 (gp130) (34). IL-6 initially attaches itself to the α subunit IL-6R on the targeted cell.

After the IL-6/IL-6R complex binds to the β subunit of gp130, the transmembrane signal-transducing IL-6R, gp130 dimerizes, and IL-6 intracellular signaling begins (42). leading to the stimulation of Janus kinase/ signal transducer and activator of transcription (JAK/STAT) pathway, mitogen-activated protein kinase (MAPK) and phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathways in cell (43). (See figure 5).

Figure 5

IL-6 signaling (42)



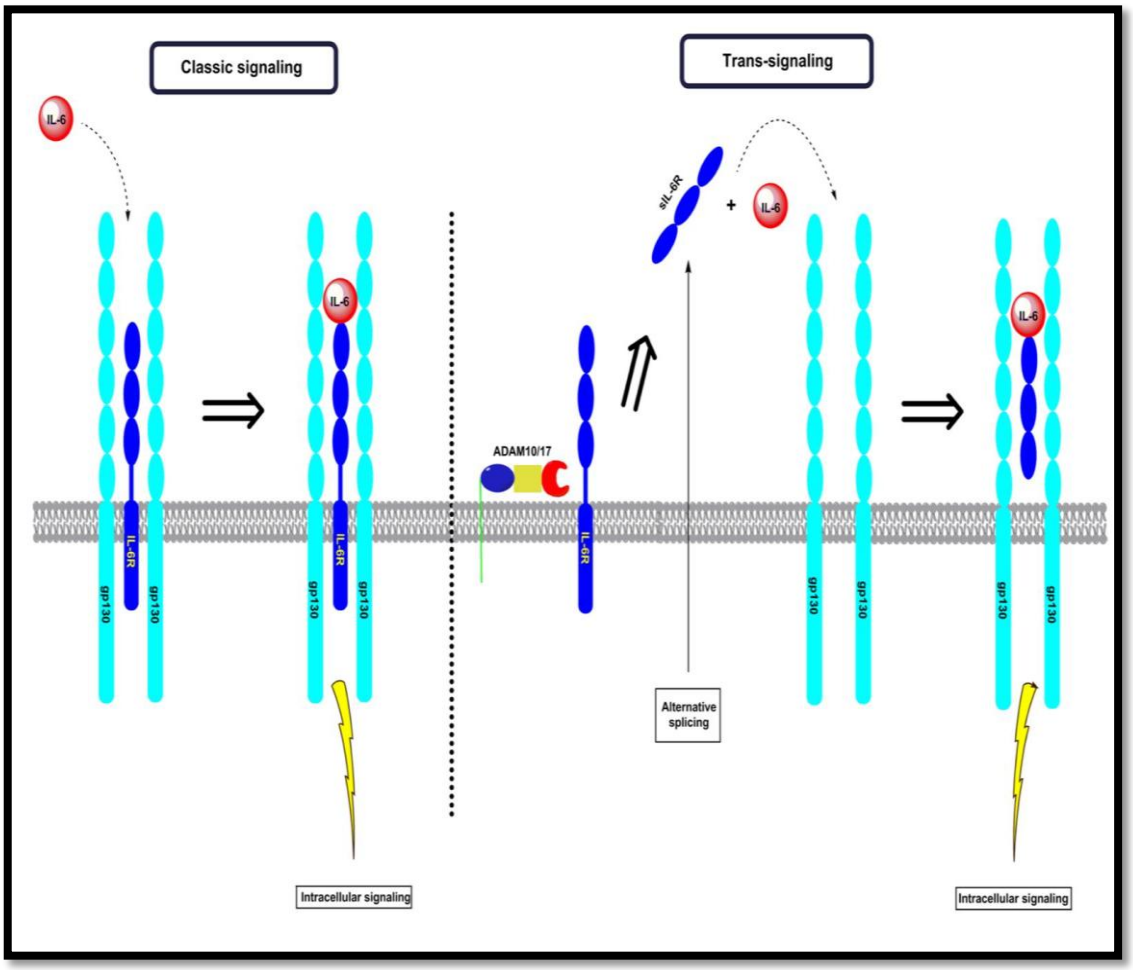
IL-6 uses two specific signaling pathways termed trans-signaling and classic signaling. In classic pathways, IL-6 directly attaches to the membrane-bound IL-6 Receptor (mIL-6R) on the cell surface, the activated gp130 subunit and the IL-6/mIL-6R complex combine to generate a signal transducing structure. Gp130 is expressed by nearly all cells throughout the body, but mIL-6R is limited to certain cell types such as

monocytes, certain epithelial cells, specific T cell subsets, and α- and β-cells within pancreatic islets. The classic pathway contributes to the anti-inflammatory effects of IL-6 (42).

In contrast, the trans-signaling pathway occurs in most cell types, which do not express mIL-6R on their surface. This can happen by binding the soluble IL-6 receptor (sIL-6R). The sIL-6R is primarily generated through shedding mIL-6R, a process facilitated by the metalloprotease enzyme A dis−integrin and metalloprotease 10 (ADAM10). It can also be translated through IL-6R mRNA. The IL-6\ sIL-6R complex can activate gp130 and start the subsequent signaling on cells lacking mIL-6R. This mechanism is known as trans-signaling because gp130 is expressed widely on nearly all cells, broadening the range of cells that IL-6 can target. Thus, trans-signaling is the driver behind the proinflammatory response (42) (see Figure 6).

Figure 6

Classic and trans-signaling of IL-6(42)



1.11 Anemia of inflammation

Anemia of inflammation, also known as anemia of chronic disease, happens because of systemic inflammation, resulting in decreased red blood cell production and life span. This anemia happens in systemic inflammatory status such as CKD, cancer or immune system (44).

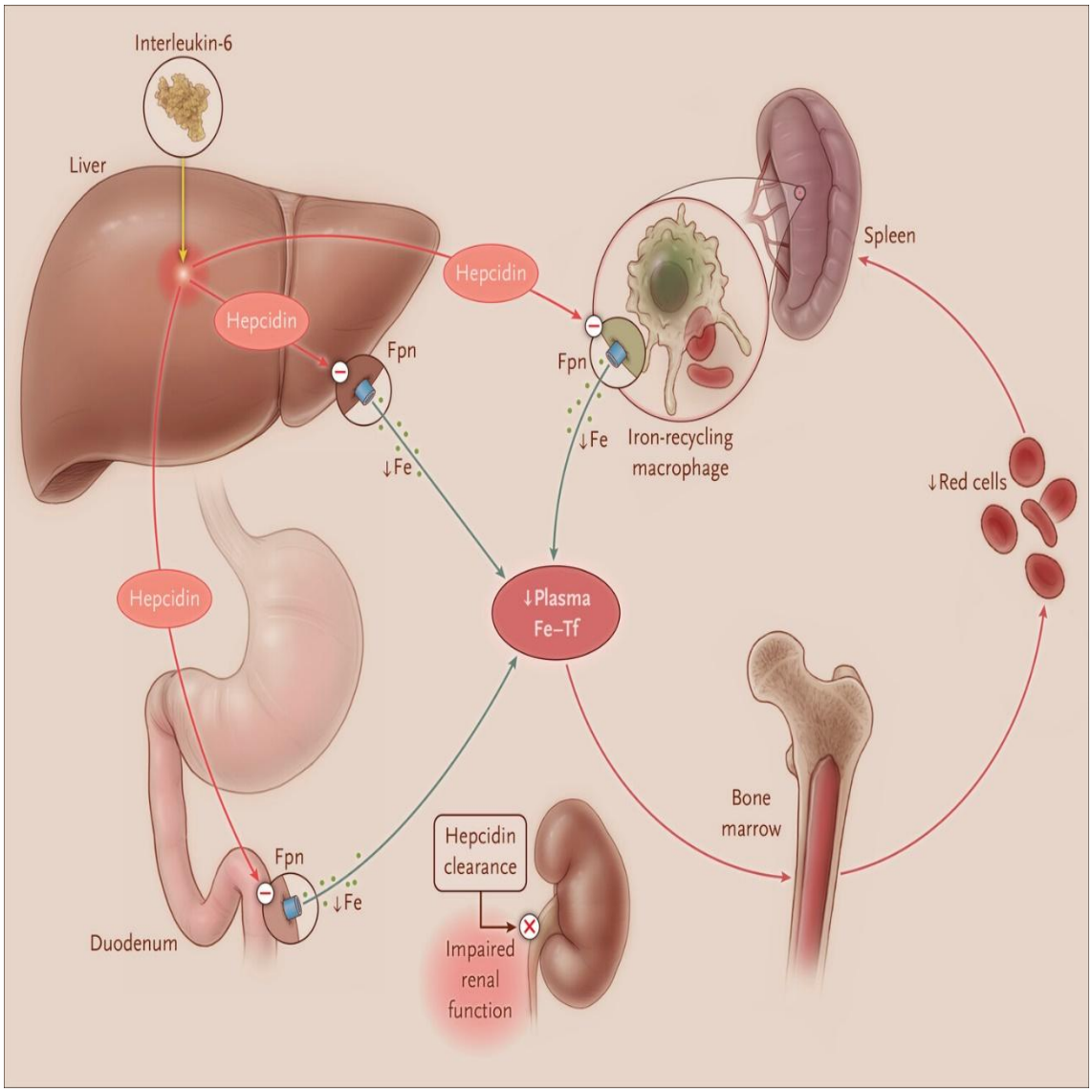
The consequences of CKD can result in the progression of anemia or inflammation. The anemia of CKD is complex and can result from reduced erythropoietin (EPO) synthesis (45), iron deficiency, and inflammation, which is a marker for CKD patients (46).

Kidneys are the primary origin of EPO, a hormone produced primarily in the kidneys and liver, and stimulates red blood cell production from bone marrow (47). Reduced EPO generation starts early when GFR drops under 30 ml/min per 1.73 m2 (48). Some patients may suffer from EPO resistance due to the direct effect of EPO, such as the negative effect of cytokines toxins on erythroid progenitor cells and decreased EPO receptors on these cells (48). Another way to EPO resistance is the upregulation of hepcidin, which can affect the proliferation and production of red blood cells (48).

High IL-6 associated with CKD patients can result in iron sequestration levels through up-regulation of hepcidin. This key regulator peptide hormone can regulate iron through decreased intestinal iron absorption (46) since iron is a rate-limiting step in red blood cell synthesis. This can reduce hemoglobin levels and anemia (49) (See Figure 7).

Figure 7

Iron hemostasis in anemia of inflammation(44)



1.12 Problem statement and rationale

Hip fractures are four times more likely among men and women on hemodialysis due to irregular bone turnover leading to osteoporosis and osteopenia, so monitoring bone mineral density in this population is very important (50).

Determining the relationship between IL-6 and bone density is essential for identifying the markers for bone quality and probable target effect.

1.13 Study significance

The incidence of ESRD patients is increasing worldwide. In addition, bone fractures are four times more likely among men and women on hemodialysis. Therefore, those patients require additional care and monitoring for their bone quality. Using IL-6 as an early, rapid and easy marker for bone loss can delay or even avoid many consequences of osteoporosis, such as fractures, which is common in dialysis patients.

Furthermore, there is an insufficient published data on the CKD patients. In the West Bank, Palestine, this was the first study which looked into the relationship between IL-6 bone density in CKD patients.

1.14 Objectives

1.14.1 Main Objective

This work tries to find out the:

- Association between IL-6 and the severity of bone mineral disease on DEXA scan.
- Measure IL-6 as a new marker for bone remodelling and reabsorption in renal osteodystrophy patients as determined by bone mineral density (BMD) at different sites.
- Evaluate the bone mass density in hemodialysis patients using DEXA scan method to determine if there is a correlation with clinical or biochemical parameters.

1.14.2 Secondary Objective

- Association between IL-6 and ferritin as an important inflammatory marker.
- Association between IL-6 and hemoglobin level.
- Association between IL-6 and PTH, which regulate bone turnover.
- Association between IL-6 and CVDs.

1.15 Research Questions

The researcher aims to answer the following main question:

What is the nature of the connection, if any, between IL-6 and BMD?

How does IL-6 relate to BMD, if at all?

1.16 Hypothesis

1.16.1 Alternative non-directional hypothesis

There is a statistically significant correlation between interleukin-6 (IL-6) levels and bone mineral density (BMD) at $\alpha = 0.05$ in the study population.

1.16.2 Null hypothesis

There is no statistically significant correlation between interleukin-6 (IL-6) levels and bone mineral density (BMD) in the study population (H_0: $\rho = 0$, $\alpha = 0.05$)

1.17 Literature Review

1.17.1 Correlation between IL-6, inflammation and renal disease

The researcher Pecoits-Filho examined the association between IL-6 and CRP in two groups of patients classified based on their GFR levels, emphasizing the link between inflammation and kidney function. This study enhances our understanding of how inflammation, as reflected by IL-6 levels, is related to instances of low GFR, he found that IL-6 plasma level is elevated in patients with lower GFR than others who have higher GFR. These findings indicate that a low GFR is intrinsically linked to an inflammatory state (51).

To find the correlation between IL-6 as an inflammation marker and mortality rate with AKI among these patients, a prospective study was made on COVID-19 patients, most of them suffering from CKD, compared to a control group who are CKD patients but not suffering from COVID-19. Previous research found that high IL-6 combined with COVID-19 patients correlates with increased mortality. In addition, 12.7% of Covid-19 patients experienced AKI upon hospitalization (52). Seminal contributions have been made by other work revealed that IL-6 is 42.1 times higher in patients who died compared to those who survived (53).

In 2017, a study on orthognathic surgery patients found that IL-6 and inflammatory cytokines and factors, in addition to the RANKL pathway, are stimulated 3-7 days after surgery (54).

A series of recent studies has indicated that IL-6 plays an important role in the progression and bad prognosis in CKD patients (55), (56) (40). Previous study have

mentioned that a high IL-6 level is correlated with increased renal endothelial permeability, impaired podocyte function, glomerular hypertrophy, enhanced fibronectin, and enhanced IL-6 mRNA expression (40).

Prior research suggests that hemodialysis patients have their own considerations regarding IL-6, CRP, and IL-6 levels, which are high in 30–50% of hemodialysis patients (57). Another study found that IL-6 in hemodialysis or pre-dialysis patients is an independent factor that can predict mortality in chronic kidney disease patients (58).

Ferritin, a positive acute phase reactant, was found to have no significant association with high IL-6 in an Indonesian article on COVID-19 patients (59). Tsai et al. imply that patients with bad kidney outcomes had ferritin levels greater than 288 ng/mL (60).

This is in contrast to a study on CKD patients, which indicates that ferritin is increased in those patients due to inflammation (47). A recent survey conducted in 2023 suggests that low and high plasma levels of ferritin are associated with bad prognosis in kidney state for those patients, independent of other factors (60).

1.17.2 Acute Kidney Injury, renal disease and IL-6

According to a study on mouse\//, AKI and IL-6 expression were closely correlated. It was discovered that following 60 minutes of bilateral kidney ischemia in an ischemic AKI mouse model, IL-6 expression and signaling are enhanced locally and systemically. This result demonstrated the relationship between systemic and local inflammation and suggested the potential use of IL-6 signaling as a biomarker and treatment target in ischemic AKI (61).

Another study revealed that IL-6 levels increased in renal fibrosis after AKI (62). In addition, IL-6 serum levels can be used as a predictor of clinical outcomes, prognosis, and mortality of AKI patients admitted to intensive care units in Japan (63).

In addition, another study indicates that elevated IL-6 plasma levels in CKD patients are not only an outcome of CKD disease but also, more crucially, a trigger for the development of CKD and its associated complications (55).

1.17.3 Bone density in chronic kidney disease

BMD can predict bone fractures in CKD patients with high specificity and moderate sensitivity (64). Low BMD is independently correlated with stages 3 to 5 of fractured patients with CKD, neither dialysis nor pre-dialysis (65). In addition, BMD can identify the presence of fractures in pre- and post-dialysis CKD (66).

Based on a Palestinian study, it was found that ESRD patients had a high prevalence of osteopenia and osteoporosis at the hip and spine. In addition, these results on low BMD patients are associated with high serum PTH, which is an indicator of low BMD (4). It was also found that long kidney dialysis duration is also correlated with high osteoporosis and low BMD (4).

Bone abnormalities like osteoporosis and osteopenia were seen in 42.8 % and 40.2 % of end-stage renal disease patients, respectively. Fractures are four times more likely among men and women on hemodialysis (25). In parallel, most patients with 3a to 5D stage of CKD had a low BMD, which had been highly predictive for fracture risk and high mortality (67).

1.17.4 Bone density in kidney disease and its correlation with IL-6

In 1996, a study examined the mRNA expression of IL-6 and IL-6R in the bone as well as the relationship between bone cell activity in dialysis patients. It found increasing IL-6R mRNA expression in osteoclasts, suggesting a link between these osteoclasts and bone remodeling, especially in individuals with excessive bone resorption (68). Another study on rodents demonstrates that serum level of IL6- correlates with a biochemical marker of bone resorption (69).

A previous study in 2001 of 137 postmenopausal women in Germany, 52–80 years old, looked into the effect of serum IL-6 as a prognostic marker of bone loss. It reveals that serum IL-6 can predict femoral bone loss in postmenopausal women (53). Another study also confirmed an association between IL-6 −174 G/C gene polymorphism and osteoporosis, which is defined as having very low BMD in postmenopausal women (70). In parallel, another meta-analysis study showed that 174C and G-634C gene polymorphism linked to a high BMD at the femoral neck, distal radius, and distal radius, respectively (71).

A recent study in 2016 on the impact of glucocorticoid-induced osteoporosis on mouse models was established, and it was found that these mouse models have problems with osteogenesis and bone metabolism and higher IL-6 level release compared with the control model. This study predicts that IL-6 could be a predictive value for fracture risks (72).

Although Studies are required to determine the precise function of the cytokine in the illness and if elevated levels represent a pathogenic trigger or a sign of, for instance, decreased renal clearance of the cytokine because of the decreased glomerular filtration function linked to kidney impairment (40).

1.17.5 IL-6 with laboratory parameters

A prospective study on ESRD patients on dialysis considered albumin a negative phase reactant that decreases as a response to inflammatory stimuli. It was also mentioned that IL-6 can predict low serum albumin and mortality in those patients (73). Similarly, IL-6 is inversely proportional to serum albumin in patients with a PTH level of 300-600 pg/mL (74). In Inker, L. A study, which include 42985 CKD patients, 58% of participants had PTH serum level of high than 65 pg\ml (75).

1.17.6 IL-6 in CKD patients and its effect on hematological parameter

A study mentioned that an inflammatory state linked to CKD could elevate hepcidin and ferritin as an acute phase reactants regardless of the iron level in the body, even in anemic CKD patients who exhibit low EPO and iron serum (47).

A systemic review on the role of IL-6 in chronic kidney disease in MEDLINE databases found that Interleukin-6 plays an essential role in the progression of chronic disease anemia via various inflammatory conditions such as end-stage renal disease (20).

1.17.7 IL-6 and CVDs

CVDs are considered the primary cause of global mortality(76). CVDs origin is complex, and this is due to the combination of environmental and genetic factors. Nowadays, inflammatory processes are considered crucial in the pathogenesis of CVDs (64). One of these inflammatory processes is IL-6. This is supported by some studies

which indicate that high IL-6 correlates with elevated CVD incidence in patients with CKD (77),(78),(28).

IL-6 accelerates the progression of CKD by aggravating kidney injury and initiating its complications, especially chronic vascular disease (CVD) (29). In 2021, a cohort study of 14611 patients with CKD and chronic coronary syndrome was established and searched for the link between IL-6 and cardiovascular outcomes. Major adverse cardiovascular events are associated with high IL-6 in all CKD categories (77).

Hyperphosphatemia because of CKD can stimulate myocardium and valve calcification and is combined with a high risk of mortality and morbidity in cardiovascular patients (27). In addition, patients with renal anemia can adversely affect the myocardium due to the imbalance between myocardial oxygen supply and demand (79).

A cohort study that analyzed data from 300 hemodialysis patients in a dialysis center showed cardiovascular events were greater during the first year of dialysis than the second (80). Another research showed that IL-6 is linked to the development of Coronary Artery Calcification and death rates in hemodialysis patients, taking into account adjusting smoking, age, gender, race, and diabetes (81).

A study have shown that low BMD, as determined by DEXA, is also linked with the prevalence and degree of arterial calcification and an elevated mortality risk in ESRD patients (39).

1.17.8 CKD, IL-6 and diabetes mellitus

Diabetes is considered one of the leading cause of CKD (19), renal and kidney consequences is the primary triggers for ESRD, the consequence of diabetes is rising due to the increasing in life quality, which can increase diabetes incidence (18).

In Palestine, the prevalence of type 2 diabetes mellitus was 15.3% in 2010, and it is expected to reach 23.4 % in 2030, which is very high in comparison with global prevalence (82).

The association between IL-6 and CVDs outcomes was previously reported in a study comparing IL-6 levels on baselines and year one, 3, with (CV) cardiovascular risk and kidney outcomes in patients with type 2 DM with high cardiovascular risk. Patients with

lower CV and kidney bad prognosis are associated with lower IL-6 at bassline, and patients with higher CV and kidney bad prognosis are associated with higher IL-6 at bassline (78).

1.17.9 Vitamin D and IL-6

A narrative review published in 2021 compared vitamin D as an IL-6 immunomodulator and tocilizumab as an IL-6 antagonist. This study found that vitamin D inhibits the production of IL-6 by immune cells, thus lowering pro-inflammatory effects. However, vitamin D does not directly target IL-6R. Thus, vitamin D may be preferable and safer than tocilizumab (47).

In 2002, a study conducted on 28 hemodialysis patients showed that IV calcitriol treatment raises bone mineral density with an observed fall in IL-6 serum level (71).

1.17.10 New potential therapeutic medicine targeting IL-6

Monitoring of interleukin concentrations in patients can be employed as a diagnostic marker for the onset or progression of various diseases, including renal diseases (83).

IL-6 has emerged as a main target in medical assessment. recent study has worked on IL-6 as a pro-inflammatory cytokines, as IL-6 is a potential target to inhibit bone destruction and stimulate bone repair (28).

Directly targeting IL-6 for anti-inflammatory action in CKD patients is an exciting avenue to investigate. Even so, neither available IL-6 inhibitors are approved for renal failure patients, nor are clinical trials limited (17).

Chapter Two

Methodology

2.1 Study design

A cross-sectional study was conducted among ESRD patients in the An-Najah National University Hospital, Nablus, Palestine dialysis unit.

2.2 Study time and setting

The study was carried out over a span of approximately three months, from March to May 2022, at An-Najah National University Hospital in Nablus, Palestine.

The selection of this hospital was due to the significant size of its dialysis department as a refer hospital, serving a majority of dialysis patients in the North West Bank. Each patient underwent testing for IL-6 levels and bone mineral density during the study.

2.3 Study population

Participants were 138 patients with ESRD in the dialysis unit at An-Najah National University Hospital, Nablus, Palestine, who were on regular hemodialysis (3 times weekly, four hours per session) of both genders.

Patients who have any of the following were excluded:

1. Their ages are less than 18 years
2. Having a history of bone malignancy.

2.4 Sample size and Sampling technique

The sample size for this study was 138 patients. The study was conducted on all patients who meet the inclusion and exclusion criteria and are available for dialysis. This method selected the sample because of its simplicity, not cost, and is more representative of all dialysis patients in the West Bank, so this sampling technique was preferred.

2.5 Study variables

2.5.1 Dependent variables:

1. IL-6 level
2. Bone density score

2.5.2 Independent variables:

1. Age.
2. Gender.
3. BMI.
4. Fracture history.
5. Diabetes mellitus.
6. Hypertension.
7. Glucocorticoid.
8. Renal transplantation.
9. Stroke.
10. Smoking.
11. Duration of dialysis.
12. History of Ischemic heart disease (IHD).
13. Labs: albumin, calcium, ferritin, iron, phosphate, transferrin saturation, Hemoglobin, white blood cells, PTH, IL-6.

2.6 Measurement tools

Clinical and demographic information about the patient were obtained from their medical record in NNUH, which include age, gender, BMI, duration of dialysis (months), diabetic state (yes, no), hypertension state (yes, no), Fracture history (yes, no), Stroke (yes, no), Smoking (yes, no) and if there is a history of Ischemic heart disease (IHD) or renal transplantation.

The results for plasma Albumin, Calcium, Ferritin, Iron, Phosphate, Transferrin saturation, hemoglobin, white blood cells and PTH were obtained from the NNUH patient file, as they are updated monthly, except for PTH, which is assessed every three months, using (Roche, Cobas C601 Germany) analyzer, in the month when the sample was collected.

For IL-6, all blood samples were collected from the patient before the start of the dialysis session in all withdrawal periods. Regarding the IL-6 Kit Guidebook from DRG GmbH instrument kit, Marburg, serum or plasma can be used for samples, and the sample can be stored at -20°C for a maximum of 2 months and at -70°C for longer storage (maximum one year). Based on that, blood samples were drawn by NNUH dialysis nursing staff using the plain tube from the patient, then the samples were transferred to the laboratory in order to separate serum using a centrifuge at 3400 rounds per minute, and the serum was frozen at -70 °C in order to preserve it until all sample is collected.

Each sample was tested for IL-6 using an enzyme-linked immunosorbent assay (ELISA) method. The same method is known by other names, such as enzyme immunoassay (EIA), a solid phase enzyme amplified sensitivity immunoassay conducted on a microtiter plate, used to detect soluble molecules such as peptides, proteins, antibodies, and hormones. A particular antibody-antigen interaction is the most important aspect of an ELISA.

The sandwich ELISA assay used in this study utilizes monoclonal antibodies coated on a microtiter plate, targeting a specific epitope on IL-6 found on samples or calibrators, along with another capture antibody labelled with horseradish peroxidase enzyme. After that, an incubation period is needed to allow complete coating and sandwich formation, and then rewashing the microtiter plate with buffer is required to remove unbounded antibodies. Substrate, a chromogenic solution, is added, and another incubation is needed. In the end, a stop solution is required in order to stop the chromogenic reaction. Elisa reader (diasource, belgium) is used as a last step to measure the absorbance. By using light at one wavelength, the amount of light absorbed reflects the substrate turnover, which is proportional to the concentration.

Bone mineral density was assessed by dual-energy X-ray absorptiometry (DEXA), a medical imaging diagnostic test, considered the gold stadnered image for predicting future fracture and for diagnosing osteopenia and osteoporosis. It is considered the test of choice for examining the strength of the bones because it is easy, performed in a short period of time (usually takes 10 to 20 minutes), inexpensive, has high accuracy and uses a low dose of radiation. It was performed at different sites of the skeletal bone

(84) because evaluating different body parts is crucial because different body parts have varied bone structures that can be affected by many variables (85).

The hip and spine are the two key sites that the DEXA scan focuses on. The International Society for Clinical Densitometry (ISCD) recommended that BMD measurements be taken of the posteroanterior spine (L1–L4) and hip. The principle of DEXA scan states that different body tissues can be distinguished based on how differently they attenuate X-rays when they pass through the body (84).

BMD measurements by DEXA offer essential predictive information on bone health and the severity of osteoporosis (86). DEXA scan results are reported as BMD (g/cm2), Z-score, or T-score. T score is represented within 1SD (+1 or -1). The more the T score is, the stronger the bones are (24). As per the standards established by the World Health Organization (WHO):

- A T-score equal to or exceeding -1.0 indicates normal bone density (24).
- A T-score falling between -1.0 and -2.5 is indicative of low bone density, commonly referred to as osteopenia (24).
- A T-score of -2.5 or below signifies the presence of osteoporosis (24).

2.7 Statistical Analysis/Analysis Plan

The Statistical Package for Social Science (SPSS version 22) was used for data entry and analysis. Frequencies and percentages were used for categorical variables, while means, standard deviations, medians and ranges were used for numerical (continuous) variables.

After assuming the hypothesis for this study, the significant level is 5%. The data was collected from the dialysis center of An-Najah National University Hospital (NNUH). Then, the hypothesis was tested by a significant test (P-value). If the P-value is less than 0.05, (there is a relationship between …), the null hypothesis is rejected, whereas when the P-value is more than 0.05, the null hypothesis is not rejected even if there is enough evidence that the null hypothesis is true.

Pearson Correlation Coefficient was used to test correlations between individual variables. The researcher also used a logistic Poisson regression model to identify characteristics related to high IL-6. The results were presented as an adjusted prevalence ratio with 95% Confidence Intervals (95% CI).

2.8 Ethical considerations

All participants received oral and written informed consent forms to participate in the study. The study was carried out with the approval of An-Najah National University's Institutional Review Board (IRB) of An-Najah National University.

The primary investigator invited the relevant subjects to participate in the study, and all agreed. Blood samples were drawn in the closed dialysis clinic of NNUH to ensure the privacy and confidentiality of the collected data.

In addition, laboratory tests have been conducted with high privacy in the NNUH and NNU. Approval to access patient data, such as patient characteristics, has been obtained from NNUH.

Chapter Three

Results

3.1 Background, clinical, and Socio-demographic status of participants

The following table (Table 1) presents the descriptive findings related to the patient's background characteristics, clinical factors, and laboratory variables. One hundred thirty-eight patients, all undergoing hemodialysis, participated in the study.

The age was categorized into two categories (greater than 25 years and younger than 25 years) to determine if there is a statistically significant relationship between the age category and other factors. The majority of the enrolled patients (66.7%) were under the age of 65, with a median age of 59 (range: 19 to 86 years).

The gender distribution is skewed towards males, constituting 60.9% of the patients.

The BMI was categorized into three categories (underweight, normal and obese). The median BMI was 27.1 kg/m2, ranging from 17.6 to 45.3 kg/m2, and the average duration of dialysis was 52.3 months (range: 1 to 240 months).

In terms of comorbidities, hypertension was the most prevalent condition among the patients (76.8%), followed by diabetes (50.7%) and IHD (47.1%).

According to fracture history, patients were asked if they had a history of bone fracture. Around 29% of patients have a previous fracture history, while 71% have not.

For CVDs, (47.1%) of patients have IHD, and (17.4%) of them have a stroke.

Table 1

Descriptive of the patients' background, Socio-demographic status, and clinical characteristics (n=138)

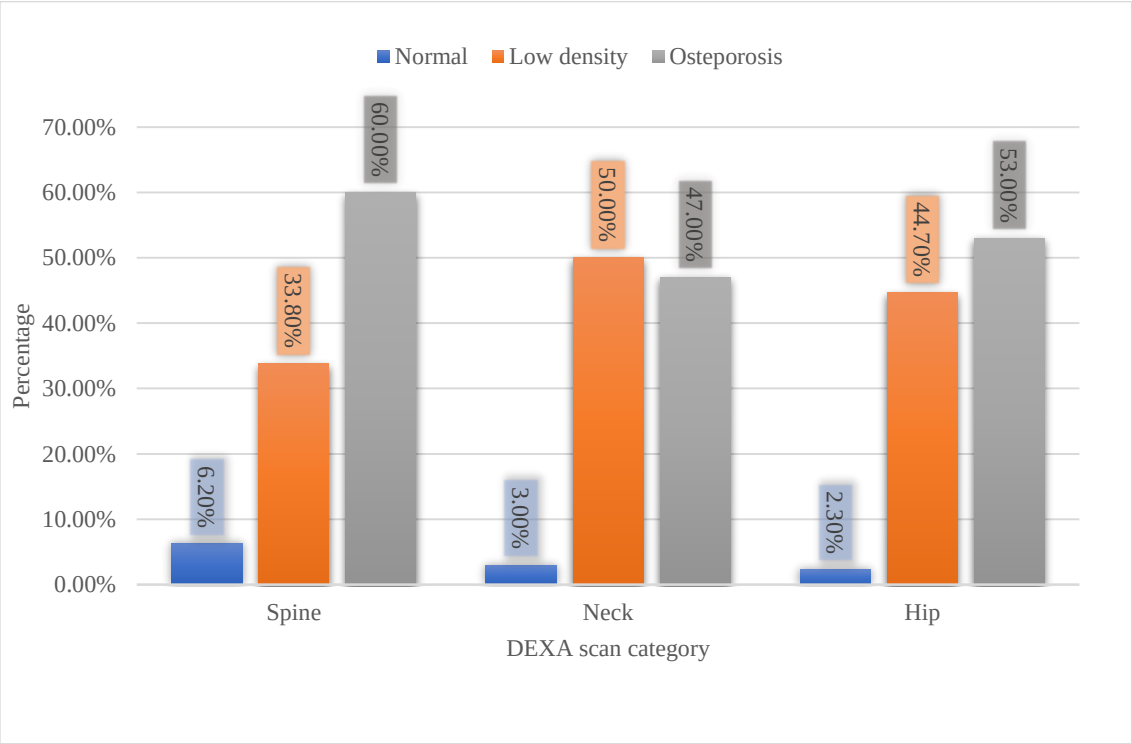
Characteristic	Frequency (%)	Median (min – max)
Age		
< 65 years old	92(66.7%)	59 (19 – 86)
≥ 65 years old	46(33.3%)	
Gender		
Male	84 (60.9%)	––
Female	54(39.1%)	
BMI	--	27.1 (17.6 – 45.3)
Dialysis duration month	--	52.3 ± 42.6 (48.0, 1-240)
Fractures history		
Yes	40 (29%)	––
No	98(71%)	
Comorbidities		
Hypertension	106 (76.8%)	--
Diabetes	70 (50.7%)	--
Smoking	52 (37.70%)	--
Glucocorticoid	23 (16.7%)	
Renal transplant	10 (7.20%)	--
Total number of comorbidities		3 (0-6)
CVDs		
IHD	65 (47.1%)	--
Stroke	24 (17.4%)	--

3.2 Distribution of bone mass density among patients

Figure 8 shows the bone mass density distribution in spine, neck and hip patients. According to the site, (60%) of patients had osteoporosis in the spine, (53%) in the hip and (47%) in the neck, whereas (50%) of patients had osteopenia in the neck, (44.7%) in the hip and (33.8%) in the spine. According to the figure below, it can be noticed that the majority of the patients have osteoporosis or osteopenia.

Figure 8

Distribution of bone mass density among patients



3.3 The average mean of Laboratory test results for patients

Table 2 details the patient's laboratory test results, utilizing means, standard deviations, and interquartile ranges (IQR) to eliminate extreme values. The average values for the laboratory tests are as follows: albumin 3.8 g/dL, calcium 8.9 mg/dL, ferritin 784.4 ng/mL, iron 64.6 ug/dL, phosphate 4.9 mg/dL, transferrin saturation (32.7%), hemoglobin 11.1 g/dL, white blood cells 16.6 K/uL, PTH 464.9 pg/mL, and IL-6 17.7 pg/mL.

From Table 2, it is seen that the mean levels for ferritin (784.4 ng/mL), phosphorus (4.9 mg/dL) and PTH (464.9 pg/mL) were higher than normal.

Table 2

Summary of the patients' laboratory findings

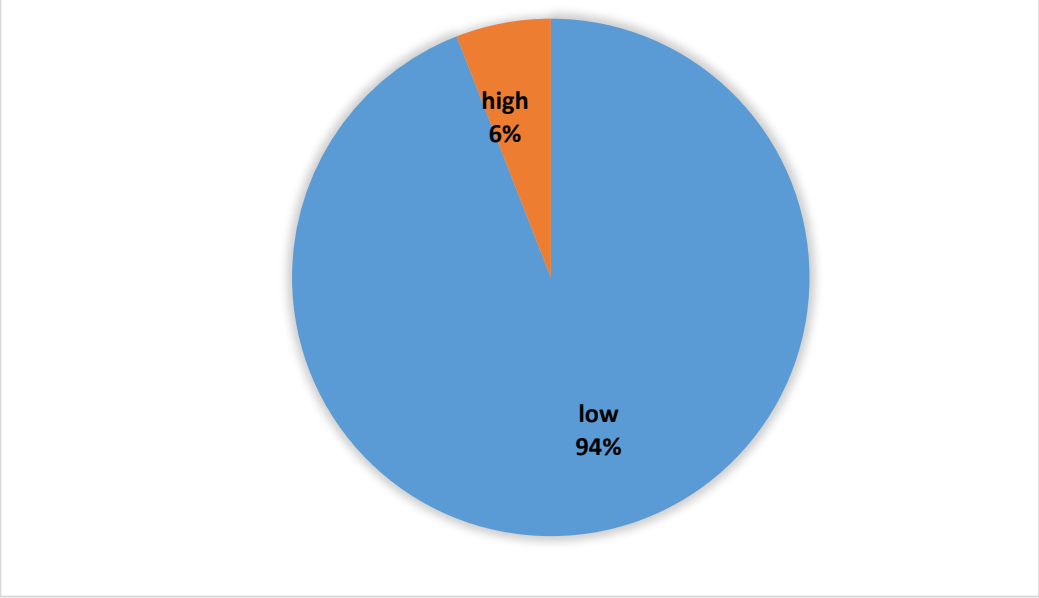
Laboratory test (normal range)	Mean ± SD (IQR)
Albumin (3.5 – 5.2 g/dL)	3.8 ± 0.4 (3.7 – 4.1)
Calcium (8.6 – 10 mg/dL)	8.9 ± 0.8 (8.50 – 9.5)
Ferritin (20 – 300 ng/mL)	784.4 ± 416.7 (472.5 – 1005.0)
Iron (50 – 160 ug/dL)	64.6± 24.4 (46.6 – 77.5)
Phosphate (2.5 – 4.5 mg/dL)	4.9 ± 1.3 (3.8 – 5.8)
Transferrin saturation (15 – 55 %)	32.7 ± 15.9 (23.0 – 38.0)
Hemoglobin (14 – 18 g/dL)	11.1 ± 2.6 (9.8 – 12.1)
White blood cells (4.5 – 10 K/uL)	6.5 ± 2.2 (5.2– 7.7)
PTH (16 – 65 pg/mL)	464.9 ± 384.3 (203.1 – 567.5)
IL-6 (0-50 pg/mL)	12.5 ± 15.6 (3.5– 15.7)

3.4 IL-6 distribution between patients

The following figure (Figure 9) represents the percentage of low and high IL-6 for patients. It shows that (94%) of the patients exhibit a low IL-6 level, defined in this study as less than 50 Pg/ml, according to the leaflet of the kit used in this study.

Figure 9

The percentage of Low and high IL-6 in patients



3.5 Analysis of correlation between IL-6 values and other variable

Table 3 indicated that there were no statistically significant differences in the distribution of IL-6 low and high values across BMI and dialysis duration month.

However, when exploring the relationship between the number of comorbidities and IL-6 values (as continuous variables), a significant and positive weak correlation was identified (r = 0.201, p-value = 0.025) (Table 5). This implies a moderate increase in IL-6 values with an elevated total number of comorbidities in patients.

Notably, when examined individually, there was no significant difference in IL-6 low or high values among the various comorbidities.

Table 3

Correlation between IL-6 values and other variable

Laboratory test (normal range)	Spearman Correlation coefficient	P value
BMI	0.1	0.214
Dialysis duration month	0.1	0.532
Number of Comorbidities	0.1	0.133

3.6 Analysis of the correlation between IL-6 and laboratory findings

Table 4 indicates a significant correlation between an elevated IL-6 level and a reduction in albumin concentration (r = -0.4, p-value < 0.001), an increase in ferritin concentration (r = 0.2, p-value = 0.011), a decrease in iron concentration (r = -0.2, p-value = 0.008), a decrease in transferrin saturation (r = -0.2, p-value = 0.013), and a decrease in hemoglobin concentration (r = -0.2, p-value = 0.018).

No significant correlations were observed between the table's IL-6 value and the other parameters.

Table 4

Correlation between patients' laboratory findings and IL-6 values

Laboratory test (normal range)	Spearman Correlation coefficient	P value
Albumin	- 0.4	< 0.001
Calcium	0.03	0.731
Ferritin	0.2	0.011
Iron	- 0.2	0.008
Phosphate	- 0.1	0.262
Transferrin saturation	- 0.2	0.013
Hemoglobin	- 0.2	0.018
White blood cells	0.1	0.472
PTH	- 0.1	0.511

3.7 Predictors of IL-6 levels with sociodemographic and comorbidities

The following table (Table 5) investigated the predictors of having high IL-6 levels and showed that most of the sociodemographic and comorbidities were not considered significant predictors for high IL-6 levels.

Compared to ages less than 65, patients over 65 were not significantly considered a predictor for high IL-6 levels. For gender, when compared to males, females were not significantly considered a predictor for high IL-6 levels.

In the same direction, for fracture history, parent hip fracture, renal transplantation, smoking, Glucocorticoid intake, Diabetes, hypertension, IHD and stroke, when compared to not having one of these variables, having the variable was not significantly considered a predictor for high IL-6 levels.

Table 5

Predictors of IL-6 levels with sociodemographic and comorbidities (Logistic regression model)

	OR	95% CI	p-value
Age			
< 65 years old*			
> 65 years old	1.7	0.5 – 6.1	0.379
Gender			
Male*			
Female	0.6	0.1 – 2.2	0.406
Fractures history			
Yes	2.1	0.6 – 7.4	0.239
No*			
Parent hip fracture			
Yes	0.98	0.1 – 8.4	0.987
No*			
Renal transplant			
Yes	--	--	0.999
No*			
Smoking			
Yes	0.9	0.3 – 3.3	0.894
No*			
Glucocorticoid intake			
Yes	--	--	0.998
No*			
Diabetes			
Yes	1.7	0.5 – 5.98	0.434
No*			
Hypertension			
Yes	1.0	0.2 – 5.1	0.98
No*			
IHD			
Yes	1.8	0.5 – 6.6	0.35
No*			
Stroke			
Yes	1.75	0.4 – 7.2	0.436
No*			

**Reference group*

3.8 Predictors of IL-6 levels with laboratory result categories

The following table (Table 6) investigated the predictors of having high IL-6 levels with laboratory result categories and showed that they were not considered significant predictors for high IL-6 levels.

On the other hand, when compared to normal values, low albumin levels were significantly considered a predictor for high IL-6 levels (p-value = 0.008), with an OR of 5.8 (95% CI = 1.6 – 21.2), which means that patients who have low albumin levels are predicted to have high IL-6 levels by 5.8 times compared to who have normal albumin values.

When compared to normal values, low iron levels were significantly considered a predictor for high IL-6 levels (p-value = 0.006), with an OR of 6.9 (95% CI = 1.7 – 27.6), which means that patients who have low iron levels are predicted to have high IL-6 levels by 6.9 times compared to who have normal iron values.

Lastly, when compared to normal values, high WBCs levels were significantly considered a predictor for high IL-6 levels (p-value = 0.009), with an OR of 8.5 (95% CI = 1.7 – 42.6), which means that patients who have high WBCs levels are predicted to have high IL-6 levels by 8.5 times compared to who have normal WBCs values.

Table 6

Predictors of IL-6 levels with laboratory result categories

		OR	95% CI	p-value
Albumin				
	High	-	-	-
	Low	5.8	1.6 – 21.2	0.008
	Normal*			
Calcium				
	High	1.5	0.2 – 13.8	0.72
	Low	1.0	0.3 – 4.2	0.969
	Normal*			
Ferritin				
	High	-	-	0.999
	Low	-	-	1.000
	Normal*			
Iron				
	High	-	-	1.000
	Low	6.9	1.7 – 27.6	0.006
	Normal*			
Phosphate				
	High	0.5	0.1 – 1.6	0.232
	Low	-	-	0.999
	Normal*			
Transferrin saturation				
	High	1.5	0.2 – 12.8	0.738
	Low	-	-	0.999
	Normal*			
Hemoglobin				
	High	-	-	1.000
	Low	-	-	0.999
	Normal*			
WBCs				
	High	8.5	1.7 – 42.6	0.009
	Low	1.8	0.3 – 9.5	0.496
	Normal*			
PTH				
	High	-	-	0.999
	Low	-	-	1.000
	Normal*			
DEXA spine				
	Low-density	0.1	-	1.000
	Osteoporosis	0.2	-	1.000
	Normal*			
DEXA neck				
	Low-density	2.5	0.2 – 32.0	0.488
	Osteoporosis	-	-	-
	Normal*			
DEXA hip				
	Low-density	2.1	0.122 – 36.96	0.606
	Osteoporosis	-	-	-
	Normal*			

**Reference group*

3.9 Analysis of laboratory test with IL-6 using linear regression

Table 7 shows that the regression model for albumin (B = - 10.654, p-value = 0.006) and ferritin (B = 0.010, p-value = 0.003) are considered significant predictors for the value of IL-6.

Therefore, the following equation can be formulated:

IL-6 = 36.696 + (-10.654 * albumin) + (0.010 * ferritin)

Table 7

Linear regression for the predictors of IL-6 with laboratory tests

Predictor	B	t	p-value	95% CI
Intercept	36.696	1.880	0.063	-1.949 − 75.342
Albumin	-10.654	-2.791	0.006	-18.209 −-3.098
Ferritin	0.010	2.758	0.007	0.003 − 0.017
Iron	-0.110	-1.138	0.258	-0.303 − 0.082
Transferrin saturation	0.043	0.233	0.816	-0.321 − 0.407
Hemoglobin	0.749	0.818	0.415	-1.065 − 2.563

3.10 Analysis of Differences in IL-6 values according to patient demographical data and the patient's clinicalcharacteristics and comorbidities

Table 8 shows no significant difference in IL-6 levels between different age or gender categories.

It also showed a significant difference in IL-6 levels between patients with IHD and those without (p-value: 0.002).

Table 8

Differences in IL-6 values according to patient demographical data and the patients' clinical characteristics and comorbidities

Characteristic	Mean ± SD	p-value
Age		
< 65 years old	10.5 ±10.8	0.221
≥ 65 years old	16.1 ±21.9	
Gender		
Male	13.0±14.3	0.252
Female	11.6 ±17.4	
BMI		
Under weight	14.6±10.9	0.601
Normal	15.1±18.2	
Fractures history		
Yes	13.6±15.1	0.208
No	11.9±16.0	
Hypertension		
Yes	12.2±14.4	0.588
No	14.6±21.8	
Diabetes		
Yes	12.1±14.1	0.751
No	12.9±17.6	
Smoking		
Yes	11.5±12.2	0.882
No	13.1±14.5	
Glucocorticoid		
Yes	10.6±9.2	0.939
No	13.1±17.1	
Renal transplant		
yes	12.8±10.6	0.308
No	12.6±16.3	
CVDs		
IHD		
Yes	15.7±18.3	0.002
no	9.7±12.8	
Stroke		
Yes	16.7±23.0	0.270
No	11.8±14.0	

3.11 Analysis of differences in IL-6 according to laboratory test and DEXA scan category

Table 10 showed that there is a significant difference in IL-6 levels in patients with albumin (p-value:0.002) and iron results (p-value:0.037).

It also showed that there is no significant difference in IL-6 levels with different DEXA scan categories (normal, low density and osteoporosis).

Table 9

Differences in IL-6 according to laboratory test and DEXA scan category

Laboratory test	Mean ± SD	P value
Albumin		
Normal	10.6± 13.2	0.002
Low	23.8 ± 23.1	
High	−−−−	
Calcium		
Normal	13.3±17.5	0.797
Low	11.3±11.9	
High	7.5±4.6	
Ferritin		
Normal	6.1±5.1	0.342
Low	7.4±−−	
High	13.0±16.1	
Iron		
Normal	10.6±11.7	0.037
Low	17.3±15.7	
High	1.3±−−	
Phosphate		
Normal	15.0±20.4	0.597
Low	18.0±22.7	
High	10.9±11.8	
Transferrin saturation		
Normal	11.9±14.7	0.138
Low	26.4±20	
High	15.9±24.0	
Hemoglobin		
Normal	10±9.7	0.898
Low	12.5±15.8	
High	9.5±−−	
White blood cells		
Normal	10.7±11.2	0.077
Low	12.3±16.3	
High	35.2±36.6	
PTH		
Normal	9.9±8.6	0.868
Low	9.5±−−	
High	12.6±15.9	
DEXA spine		
Normal	11.7±12.6	0.654
Low-density	10.1±10.3	
osteoporosis	13.6±17.8	
DEXA neck		
Normal	7.5±7.7	0.744
Low-density	11.1±11.6	
osteoporosis	14.4±19.7	
DEXA hip		
Normal	7.2±8.2	0.316
Low-density	9.7±9.1	
osteoporosis	15.1±19.6	

The following table (Table 11) shows that patients who have more CVDs significantly had higher IL-6 values (p-value = 0.005). It also shows that the mean value for IL-6 for patients who have only CVDs (16.7) is higher than those who have DM (10.6) or both CVD and DM (12.9) and are higher than those who have none of them (8.9, p-value = 0.002).

3.12 Analysis of the correlation between mean IL-6 according to Number of CVDs (IHD, stroke and PAD) and patients having CVDs or DM

This tables shows that patients who have more CVDs significantly had higher IL-6 values (p-value = 0.005), 15 it also shows that mean value for il-6 for patients who have only CVDs (16.7) than who have DM (10.6) or both of CVD and DM (12.9), and are higher than who have none of them (8.9, p- value = 0.002).

Table 10

Differences in mean IL-6 according to Number of CVDs (IHD and stroke) and patients having CVDs or DM

Variable	Values	Mean ± SD	p-value
CVD	Zero	9.8±14.3	0.005
	One	12.0±10.0	
	Two	18.7±26.2	
	Three	25.1±16.1	
CVD and/or DM	None of DM or CVD	8.9±15	0.002
	DM only	10.6±12.7	
	CVD only	16.7±19.2	
	CVD & DM	12.9±14.8	

Chapter Four

Discussion

4.1 Prevalence according to sociodemographic data and comorbidities

This work showed that the majority of the enrolled patients (66.7%) were under the age of 65, with a median age of 59, and males represented a percentage of (60.9%) of patients. According to comorbidities, hypertension was the most prevalent condition among the patients (76.8%), followed by diabetes (50.7%). This is close to a Palestinian study that discussed bone mineral density in ESRD patients, including 194 patients with a mean age of 57. It is worth mentioning that males represent (58.8%) of the patients. (52.1%) of them have diabetes, and (78.4%) have hypertension (4). Another Palestinian study searched into the prevalence of ESRD patients in the West Bank in 2013 investigated that (57.7%) of patients were males, the majority of patients (45%) were between 45 and 64 years old, and most of the patients suffered from hypertension or diabetes or both of them (27).

Moreover, the study showed no relationship between IL-6 and increased age in patients with CKD. In contrast, a recent study emphasized the relationship between the increase in IL-6 levels due to ageing and the occurrence of atherosclerosis, which explained that age could enhance IL-6 signaling from bone marrow adipocytes and vascular cells, ageing can also increase IL-6 production as a result of reduced function of mitochondria (87). Another study indicates that IL-6 remains low or normal in healthy young individuals and is regulated by hormonal and transcription factors such as sex steroid hormone in late life, and as a result of loss of inhibitory sex steroids with ageing, IL-6 is upregulated in adults (88).

The findings suggest that there is no relationship between the duration of dialysis and IL-6 level. This goes against a recent study which indicates that low BMD correlates with increased dialysis duration as a result of the gradual loss of kidney function and the consequences of CKD (4). However, in another study, the duration of dialysis was considered a strong predictor of BMD (89).

4.2 Correlation between IL-6 with CKD state and inflammatory marker

Contrary to this work's expectations, this study showed that only (6%) of our patients had high IL-6 levels. This goes against most findings and articles, which showed that IL-6 is significantly high in ESRD and CKD patients and is considered a predictor of mortality and morbidity. This can be explained by the diminished elimination of cytokines and stimulation of cytokines and oxidative stress in uremia, which are associated with chronic diseases such as diabetes, IHD and CVDs (90). Furthermore, it was found that high IL-6 is not only a result of CKD but can serve as a stimulus for the progression of CKD and its secondary effects (55).

On the other hand, another study analyzed the association between IL-6, inflammation and low GFR. It found that low GFR linked to the inflammatory state in CKD patients correlates with IL-6 levels, which could be due to the negative impact of inflammation on kidney function in addition to the increased production of cytokines along with reduced clearance from the kidney (51).

In the same scenario, IL-6 levels were high in (30%-50%) of Spanish hemodialysis patients. This is explained by the fact that hemodialysis patient experiences higher inflammatory states and cytokines related to more exposure to oxidative stress, loss of clearance of cytokines, their underlying comorbidities, and other factors related to hemodialysis technique (57).

Our study indicates that ferritin is considered a significant predictor for the value of IL-6. This may be due to the inflammatory state combined with CKD disease and hemodialysis and the role of IL-6 as a pro-inflammatory marker. In a similar scenario, ferritin, an acute phase reactant, was found to be elevated in most CKD patients due to inflammation and oxidative stress, regardless of iron body stores (47). Proinflammatory cytokines such as IL-6 can enhance ferritin production in an inflammatory state (91).

Zakarneh's study demonstrates that high WBCs levels were a significant predictor of high IL-6 levels in patients with CKD. This goes along with a study that indicates that total WBCs, monocytes, eosinophils and neutrophils positively correlate with IL-6 levels in women. This can be explained by the role of leukocytes in IL-6 production in vitro, with no clear information about the relationship between them in vivo (92).

As for AKI, IL-6 levels were found to be high in patients who suffered from AKI due to the induction of IL-6 production after a tissue injury and renal fibrosis due to its role in inflammation and acute phase reactant and immunological reaction (62).

4.3 Correlation between bone density with IL-6

Our finding aims to discover new bone density indicators in hemodialysis patients, avoid bone changes, and decrease future bone fractures (85). Therefore, these patients must be carefully monitored, particularly patients with multiple risk factors (4), because the possibility of fracture corresponds inversely with BMD assessments (93).

We aimed to determine the relationship between IL-6 as a bone density indicator and the severity of bone mineral disease measured by DEXA scan. The researcher revealed that most of the patients included in Zakarneh's study had osteoporosis. The prevalence in this study is (60%), (53%), and (47%_ and for osteopenia, were (50%), (44.7%), and (33.8%) at spine, hip, and neck respectively. These results are due to secondary hyperthyroidism and bone accompanied by CKD state and CKD-MBD action. This is in parallel with a Palestinian study, which reflects that the majority of ESRD patients had extremely decreased BMD, such as osteopenia and osteoporosis, with percentages of (83%) at the total, (71.1%) at the hip and (71.1%) at the spine, Low BMD was linked to a prolonged dialysis time (4).

Another study showed that most ESRD patients had osteoporosis and osteopenia along with high PTH associated with secondary hyperthyroidism linked to CKD (85). In another study, osteoporosis affects (23%) of patients, while osteopenia affects (45%) (94).

In addition, a Korean study finds that GFR correlates significantly with BMD and suggests osteoporosis is highly common in patients with moderate to severe CKD (95).

Although this work showed no significant difference between IL-6 levels with different DEXA scan categories, it can be noticed that mean IL-6 were higher in osteoporosis patients compared to osteopenia and normal BMD patients, as previously mentioned.

A similar study compares hemodialysis patients to healthy controls and found that hemodialysis patients showed higher (CRP) C-reactive protein and IL-6 serum levels than those healthy controls, which could be due to the chronic inflammatory state

combined with hemodialysis. In addition, it indicates that osteoblastic activity markers and osteoclastic activity markers were significantly increased in hemodialysis patients (96).

In parallel, a study conducted on AKI and CKD patients found that IL-6 is the root cause for the increase in FGF-23 transcription, which is linked to the low BMD associated with CKD and to high mortality and morbidity in those patients (36).

Another study showed that IL-6 is a predictor for femoral bone loss in menopausal women, especially at the first yeast of menopause, due to its role in bone remodeling and may be attributed to the sensitivity of bone cells to IL-6 at the early menopause years (97).

4.4 Albumin level with IL-6 level

Consistent with previous studies, this work showed that low albumin levels significantly predicted high IL-6 levels (p-value = 0.008). This finding aligns with a study which showed a significant relationship between hypoalbuminemia and high IL-6 in hemodialysis patients (73).

A study that supports the results of this work showed that a decrease in albumin in hemodialysis patients can be linked to an increased mortality risk by (47%), explained by inflammation (98). Hypoalbuminemia is considered a marker in dialysis patients (74). In ESRD patients, it can be explained by the imbalance between the synthesis and elimination of albumin through the kidney. This imbalance is due to the protein loss during hemodialysis, which reduces the synthesis of albumin through the liver (99).

Another reason for low albumin is that during the inflammation cascade, cytokines like IL-6 induce the liver to increase the production of acute-phase reactant proteins such as CRP. By contrast, it can reduce the synthesis of albumin through the liver.

Along with these results, a study investigated the relationship between serum albumin and IL-6 as an inflammatory mediator among the PTH group. They found that albumin is significantly lower in patients with a PTH level of more than 600 pg/mL than in other PTH groups with a lower level of 600 pg/mL. It also found an inverse correlation between serum albumin and IL-6 in patients with 300-600 pg/mL of PTH level (66).

These results can be explained by the effect of albumin on OPG bone markers in those patients, and the role of PTH in bone remodeling and its relation with the hemodialysis inflammatory process (74).

4.5 Correlation between IL-6 and anemia of chronic disease

Anemia of chronic inflammation is considered a common form of anemia. It has many contributing factors, such as the loss of iron through hemodialysis, iron sequestration and decreased renal synthesis of erythropoietin, as the researcher has discussed above (48).

In comparison with this work result and the previous research about anemia of CKD, it is worth mentioning that there is a significant correlation between an elevated IL-6 level and a decrease in iron concentration ($r = -0.2$, p-value = 0.008), a decrease in transferrin saturation ($r = -0.2$, p-value = 0.013), and a decrease in hemoglobin concentration ($r = -0.2$, p-value = 0.018). This is in agreement with a study which showed that IL-6 play a central role in the pathogenesis of anemia of chronic disease, including end-stage renal disease, via increased hepatic hepcidin, thus changes in iron levels which is the rate-limiting step in hemoglobin synthesis (20).

In addition, due to this vital role in mediated anemia, IL-6 could be a targeting agent in treating patients suffering from anemia of chronic disease (20). This is similar to a study on juvenile CKD, which links high IL-6 serum level, development of renal fibrosis and high hepcidin, leading to decreased iron that affects the erythropoiesis axis (100).

Another study revealed that hemodialysis patients had decreased ferroportin, the iron exporter, and this is linked to inflammation associated with hemodialysis, high hepcidin, and low iron (101).

This agrees with a study that mentioned that patients with chronic inflammation have lower serum iron, transferrin and higher ferritin than those without inflammation. That inflammation and pro-inflammatory cytokines explain that they can affect iron hemostasis through their effect on ferritin synthesis and plasma iron (20).

4.6 CKD and its correlation between IL-6 and IHD

This study showed a significant difference in IL-6 levels between patients with IHD and those without. This is linked with patients with ESRD, who have a much greater incidence of IHD than the general population, according to a 2020 study that looked at a cohort of 2000–2010 ESRD patients. Most of this happened within three years of beginning hemodialysis, in contrast to the overall population (102).

A previous study showed that patients with IHD who experienced elevated IL-6 levels had an 8.6-fold higher risk of mortality from cardiovascular causes within six years Compared to individuals with low IL-6 values (103).

4.7 CKD and its correlation with IL-6 and CVDs

This work showed that patients with more CVDs significantly had higher IL-6 values (p-value = 0.005). This is linked with a comparative analysis study, which investigates that IL-6 level may be the most accurate indicator of CVD and mortality in patients with ESRD among these biomarkers (Albumin, CPR, IL-6, and Fetuin) (104).

Another meta-analysis study summarized 11 studies indicating that IL-6 serve as a predictor of CVDs risk in healthy patients. Individuals who eventually developed CVD during the follow-up period exhibited significantly higher IL-6 levels compared to those who did not experience CVD, among others, which can be due to the inflammatory pathways (105).

A meta-analysis study studied the role of IL-6 in mortality and morbidity in dialysis patients caused by CVDs, indicating that cardiovascular mortality is higher for patients with high IL-6 serum levels. This could open a new way to target IL-6 to help a patient have a good prognosis and monitor dialysis (106). The IL-6 can initiate cardiovascular events through its signaling pathway, JAK/STAT and mitogen-activated protein kinase. This can lead to atherosclerosis and other myocardial events (106).

It also found that IL-6 can facilitate the occurrence of endothelial injury through the JAK/STAT pathway by decreasing adiponectin (an anti-atherogenic adipokine) expression and endothelial nitric oxide synthase (55), which is an enzyme responsible for nitric oxide synthesis in endothelial cells to maintain vascular hemostasis, which leads to endothelial dysfunction (107).

Another way in which IL-6 can lead to sudden cardiac death is that IL-6 can influence electrical cardiac equilibrium through its direct effect, which can result in tachycardia and an imbalance in heart rate (108).

A similar scenario was carried out and predicted a strong association between high IL-6 with bad kidney and CV outcome, which can be explained by the role of IL-6 on the white blood cells and the formation of atherosclerotic plaque, and its role in fibrosis in the kidney as a result of generation of reactive oxygen species.

https://pmc.ncbi.nlm.nih.gov/articles/PMC9862371/pdf/dc220866.pdf

4.8 CKD and its correlation between IL-6, DM and CVDs

This study indicates that those having CVD or DM or both can have higher IL-6 levels than patients who have none of them, which is may be due to the mIL-6R, which is found in pancreatic cells and the direct role of IL-6 on pancreatic cells and insulin sensitive tissue, which may suffer in insulin resistant or malfunctioning of the beta cell (42).

Furthermore, a Palestinian study conducted in the West Bank among diabetic patients revealed that 23.6% of them were diagnosed with CKD. The research identified smoking, hypertension, and age exceeding 65 years as factors that markedly elevate the likelihood of CKD development in diabetic individuals (1)

It has long been hypothesized that the pathophysiology of type 2 Diabetes mellitus is primarily driven by systemic chronic inflammation, which is mediated by pro-inflammatory cytokines like IL-6. Moreover, Diabetes mellitus (DM) is well recognized as a primary factor contributing to CKD on a global scale, responsible for around 30% to 50% of cases (109). High IL-6 levels could be due to excess adipose tissue, which can alter glucose metabolism, leading to diabetes (110).

This is similar to a study which indicates that patients with Type 1 DM have high levels of IL-6 (111). In addition, another one suggests that serum IL-6 levels were shown to be increased in type 1 Diabetes mellitus patients, and these levels were not affected by age, ethnicity, or the length of the disease (1).

In 2022, a study revealed elevated IL-6 levels in patients with diabetic nephropathy in contrast to those without the condition, indicating a potential association between IL-6 and an increased susceptibility to developing diabetic kidney disease (25). Another study, also conducted in 2022, demonstrated a significant correlation between heightened levels of IL-6 and NF-B in the blood of diabetic nephropathy patients, suggesting an association with elevated kidney injury and an unfavorable prognosis for these patients (26).

Furthermore, early intervention to manage hyperglycemia has been demonstrated to significantly lower the risk of CKD in individuals with diabetes(112). In addition, Research links the IL-6 inflammatory properties to the etiology of CVDs, CKD, and diabetes. Therefore, there may be a therapeutic benefit to targeting the IL-6 pathway in certain cardiometabolic diseases (110).

4.9 Limitation

This study has several limitations. The findings of this work could not be applied to the entire hemodialysis population in Palestine because it is conducted in one single center, NNUH dialysis unit, and because of its small sample size due to the limited budget.

Another limitation is that it is a cross-sectional study, thus the data was collected at one time, which may bear bias. In addition, this study needs to add more inflammatory marker such as CRP, this test could have been added but it could require a higher budget than what is available.

Although DEXA is a commonly used technique for quantifying BMD in CKD patients, it has some limitations. First, DEXA measures a real BMD (g/m^2), not volumetric BMD (g/m^3), meaning that the DEXA scan gives a two-dimensional rather than a three-dimensional image, providing less information about bone quality or bone structure. Second, it cannot differentiate between spongy and compact bone. In addition, it cannot assess bone remodeling or microarchitecture (24).

4.10 Recommendation

Our finding suggests low bone density in CKD patients and its correlation with IL-6 as an inflammatory marker and laboratory tests. It also highlights the relationship between IL-6, CVDs and DM.

It is recommended that an alkaline phosphatase test be added to assess the severity of bone disease and bone turnover. In addition, it is recommended that the relationship between IL-6 and CRP be studied as an inflammation marker to yield more accurate results. Moreover, it is recommended to measure IL-6 test using automated device such as architect or cobas, to obtain much accurate result and to avoid lab error from contamination, improper washing.

It is also recommended to improve life quality and bone health for CKD patients through monitoring bone density and conducting a periodic laboratory test to maintain parameters like albumin, PTH, and hemoglobin at normal range, which can delay or reduce complication of CKD on bone.

Anyway, although CKD is a serious complication worldwide with a high mortality, it can be preventive through public education, avoiding potential CKD risk factors, and slowing disease progression. So, it is recommended to early detection and regular monitoring of patients' heart and blood vessel health because of elevating the risk of adverse cardiovascular outcomes in patients suffering from CKD.

4.11 Conclusion

This study demonstrated that CKD Palestinian patients have low Bone density, and elevated levels of IL-6 were linked with low albumin, iron, transferrin saturation and hemoglobin, with increased ferritin concentration. It also demonstrated that CVDs could be linked to high IL-6 concentrations in those patients. The study suggests monitoring the patient's bone density and maintaining albumin, phosphorus and PTH at normal levels in order to enhance bone health.

List of Abbreviations

Abbreviation	Meaning
ADAM 10	A disintegrin and metalloprotease 10
AKI	Acute kidney injury
BMD	bone mineral density
CV	cardiovascular risk
CVDs	cardiovascular diseases
CaSR	CaSR calcium-sensing receptor
CRP	C-Reactive Protein
CKD	chronic kidney disease
CKD-BMD	chronic kidney disease-mineral and bone disorder
DEXA	dual-energy X-Ray absorptiometry
ESRD	End stage renal disease
ELISA	enzyme-linked immunosorbent assay
EPO	Erythropoietin
FGF-23	Fibroblast growth Factor 23
GFR	Glomerular filtration rate
gp130	glycoprotein 130
HSCs	hematopoietic stem cells
IL-6	interleukin-6
IL-6R	Interleukin-6 receptor
IHD	Ischemic heart disease
JAK/STAT	Janus kinase /signal transducers and activators of transcription
mIL-6R	membrane bound IL-6 Receptor
MSCs	mesenchymal stem cells
NNUH	Najah national university hospital
PTH	parathyroid gland hormone
RANK	Receptor activator of nuclear factor kappa-B
RANKL	Receptor activator of nuclear factor kappa-B ligand
sIL-6R	Soluble form of IL-6 receptor
SPSS	Statistical package for social science
WHO	World Health Organization

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Appendices

Appendix A

Informed consent

Palestine
An-Najah National University
Faculty of graduate studies

دولـــــــــــة
فلســـــــــطين
جامعة النجاح الوطنيـة
كليـة الدراسات العليا

اقرار عن دراسة
العلاقه بين الإنترلوكين-6 وكثافة العظام لدى مرضى غسيل الكلى في فلسطين.

1) **الباحثة:** سوار فلاح فايز زكارنه بإشراف الدكتور زاهر نزال والدكتور زكريا حمدان.
2) **هدف الدراسة:** تهدف هذه الدراسة الى معرفة العلاقة بين الإنترلوكين -6 وكثافة العظام لدى مرضى غسيل الكلى في فلسطين.
3) **سؤال الدراسة:** هل يوجد هناك علاقة بين بين الإنترلوكين -6 وكثافة العظام لدى مرضى غسيل الكلى في فلسطين؟
4) **نتائج البحث:** سوف تستخدم نتائج البحث للأغراض العلمية فقط وذلك للحصول على درجة الماجستير في الكيمياء الحيوية السريرية/ جامعه النجاح الوطنية.
5) **طريقة البحث:** ستتم من خلال سحب عينة دم بالإضافة لإجراء صورة أشعة لفحص كثافة العظام لدى المرضى.
6) **المخاطر المتوقعة:** لا يوجد أي مخاطر، حيث ان عملية سحب الدم هي عملية روتينية للمرضى وآمنه تماما، أما فحص كثافة العظام فهو عباره عن صورة أشعة تستخدم جرعه قليلة من الأشعة والتي لا تسبب أي أخطار تذكر، بالإضافة إلى انها سهله وسريعة (15-20دقيقه). المشاركة في هذه الدراسة عبارة عن عمل تطوعي ويمكن الامتناع او الانسحاب عن المشاركة في أي وقت من دون ذكر الأسباب وبدون أي التزامات أو فقدان مزايا.
7) **الاستفادة المتوقعة للمشاركين:** سيتمكن المرضى المشاركين في هذه الدراسة من معرفة نسبة كثافة العظام بالإضافة لمعرفة نتيجة فحص الإنترلوكين-6 لديهم، أما الفائدة غير المباشرة فتتضمن معرفة فيما إذا كان هنالك علاقة بين الإنترلوكين-6 وكثافة العظام لدى مرضى غسيل الكلى والذين هم بالضرورة معرضون لخطر هشاشة العظام أكثر بأربعة أضعاف من غيرهم ، بالتالي استخدام فحص الإنترلوكين لفحص وتتبع كثافة العظام بدلاً من صورة الأشعة لفحص كثافة العظام.
8) **السرية واحترام الخصوصية:** المعلومات سوف تستخدم لأغراض البحث العلمي فقط بما يضمن الحفاظ على الخصوصية والسرية التامة بحيث لا يكون هناك أي إزعاج للمشاركين . وأي استفسار او سؤال له علاقه بهذه الدراسة يمكن للشخص المشارك مراجعة الدكتور زاهر نزال(0599545421) او الدكتور زكريا حمدان (0592346486) كما يحق لاي مشارك رفض دخول الدراسة في اي وقت من الدراسة. **كل المعلومات التي سوف يتم الحصول عليها من هذا الاستبيان هي سريه وليست للنشر.** شاكرين لكم مشاركتكم وتعاونكم البناء لما فيه من الخير.

موافقة المشارك: .....

تاريخ الاجابه:..... التاريخ :.....

Appendix B

Approval from the Faculty of Graduate Studies

An-Najah
National University
Faculty of Graduate Studies
Dean's Office

جامعة
النجـــاح الوطنية
كلية الدراسات العليا
مكتب العميد

التاريخ: 2021/10/14

حضرة الدكتور اياد العلي المحترم
منسق برنامج ماجستير الكيمياء الحيوية السريرية

تحية طيبة وبعد،

الموضوع : الموافقة على عنوان الاطروحة وتحديد المشرف

قرر مجلس كلية الدراسات العليا في جلسته رقم (410) المنعقدة بتاريخ 2021/10/7، الموافقة على مشروع الأطروحة المقدم من الطالب/ة سوار فلاح فايز زكارنة، رقم التسجيل 11952296، تخصص ماجستير الكيمياء الحيوية السريرية، عنوان الأطروحة:

العلاقة بين الأنترلوكين -6 وكثافة العظام لدى مرضى غسيل الكلى في فلسطين
The Correlation between Interleukin-6 and Bone Mineral Density among Hemodialysis Patients in Palestine

بإشراف: 1) د. زاهر نزال 2) د. زكريا حمدان

ملاحظة: لاعتماد الأطروحة وتسجيلها على الفصل الاول 2022/2021.

يرجى اعلام المشرف والطالب بضرورة تسجيل الاطروحة خلال اسبوعين من تاريخ اصدار الكتاب. وفي حال عدم تسجيل الطالب/ة للاطروحة في الفترة المحددة له/ا ستقوم كلية الدراسات العليا بإلغاء اعتماد العنوان والمشرف

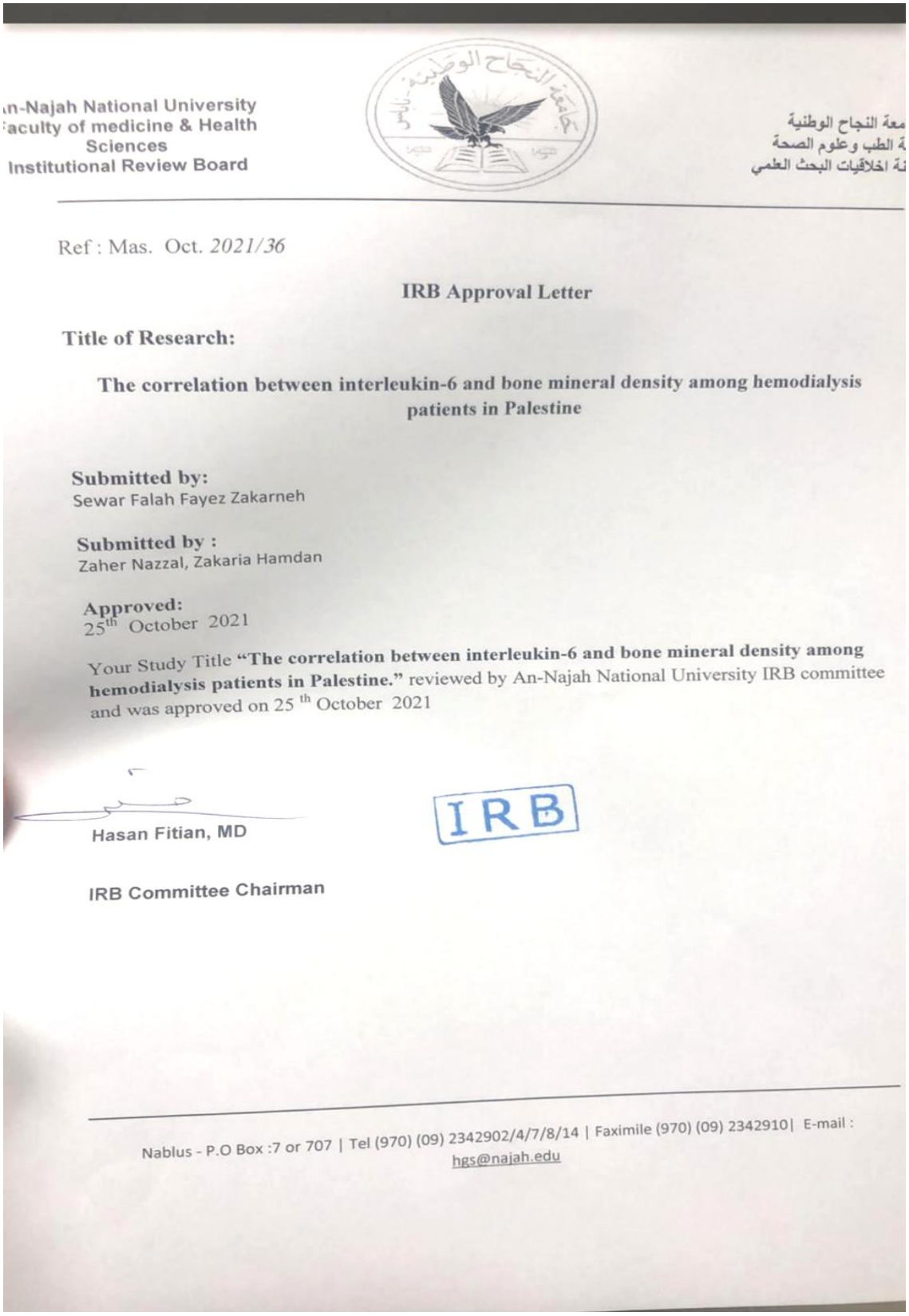
وتفضلوا بقبول وافر الاحترام ،،،

عميد كلية الدراسات العليا
أ.د. وليد صوالحة

نسخة : د. رئيس قسم الدراسات العليا للعلوم الطبية والصحية المحترم
: عميد القبول والتسجيل المحترم
: مشرف الطالب

Appendix C

IRB Approval



An-Najah National University
Faculty of medicine & Health Sciences
Institutional Review Board

جامعة النجاح الوطنية
كلية الطب وعلوم الصحة
لجنة اخلاقيات البحث العلمي

Ref : Mas. Oct. *2021/36*

IRB Approval Letter

Title of Research:

The correlation between interleukin-6 and bone mineral density among hemodialysis patients in Palestine

Submitted by:
Sewar Falah Fayez Zakarneh

Submitted by :
Zaher Nazzal, Zakaria Hamdan

Approved:
25th October 2021

Your Study Title **"The correlation between interleukin-6 and bone mineral density among hemodialysis patients in Palestine."** reviewed by An-Najah National University IRB committee and was approved on 25 th October 2021

Hasan Fitian, MD

IRB

IRB Committee Chairman

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Appendix D

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Certificate of English Proofreading and Editing

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كلية الدراسات العليا

العلاقة بين الإنترلوكين -6 وكثافة العظام لدى مرضى غسيل الكلى في فلسطين

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قدمت هذه الرسالة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء الحيوية السريرية، من كلية الدراسات العليا، في جامعة النجاح الوطنية، نابلس – فلسطين.

2024

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الملخص

المقدمة: فشل الكلى المزمن هو مشكله صحية عالمياً، وله تأثيرات على الجسم، وهذا يشمل أمراض القلب والأوعية الدموية، مشاكل أمراض الدم، وأمراض اضطراب العظم والمعدني في فشل الكلى المزمن. تعتبر متابعة كثافة العظام لدى هؤلاء المرضى أمراً وأساسياً. هدف هذه الدراسة هو تحديد العلاقة ما بين الإنترلوكين–6 وباقي الفحوصات الطبية مع كثافة العظام في مرضى فشل الكلى المزمن.

الهدف: تهدف الدراسة إلى إيجاد العلاقة ما بين الإنترلوكين–6 ومدى هشاشة العظام لدى مرضى فشل الكلى المزمن عن طريق مقياس امتصاص الأشعة السينية ثنائي البواعث، لقياس الإنترلوكين–6 كمؤشر حيوي للعظام وامتصاصها في مرض الحثل العظمي كلوي المنشأ والذي يتم تحديده اعتماداً على كثافة العظام لدى هؤلاء المرضى.

طرق البحث: تعتبر هذه الدراسة دراسة مقطعية على مرضى الفشل الكلوي المزمن في مستشفى النجاح الوطني الجامعي–نابلس، فلسطين. تم فحص نسبة الإنترلوكين الموجود في مصل ١٣٨ مريض باستخدام تقنية إليزا ، تم قياس كثافة العظام لدى المرضى باستخدام مقياس امتصاص الأشعة السينية ثنائي البواعث. تم تحليل البيانات باستخدام برنامج التحليل الإحصائي.

النتائج: تشير النتائج إلى أن معظم المرضى المشاركين في الدراسة كانوا يعانون من قلة العظام ، بنسب انتشار بلغت 60%، 53%، 47% على التوالي في الفقرات، الورك، والعنق. كما كانت نسب

الهشاشة العظمية تبلغ 50%، 44.7%، و 33.8% في نفس المواقع. نسبة الإنترلوكين-6 في مصل المرضى كانت طبيعية، مما يعني عدم وجود علاقة ما بين الإنترلوكين-6 وكثافة العظام. على الناحية الأخرى، يوجد علاقة مهمة ما بين الإنترلوكين-6 ونسبة الألبومين، مخزون الحديد، الحديد، ونشبة تشبع الحديد، ونسبة الهيموجلوبين في الدم. النتائج أظهرت أيضاً بأن المرضى الذين يعانون من أكثر من عامل من عوامل مرض القلب والتروية الدموية لديهم نسبة أعلى من الإنترلوكين-6. وجدت الدراسة أيضاً بأن المرضى الذين يعانون من مرض القلب والأوعية الدموية لديهم نسبة أعلى من الإنترلوكين-6 مقارنة بالمرضى الذين لديهم مرض السكري أو كلى المرضي.

الخاتمة: أظهرت الدراسة بأن مرضى الفشل الكلوي المزمن الفلسطينيين لديهم نقص في كثافة العظام، و وجود علاقة قويه ما بين ارتفاع الإنترلوكين-6 و وانخفاض نسبة الألبومين، الحديد، نسبة تشبع الحديد، ونسبة الهيموجلوبين في الدم، وارتفاع مخزون الحديد وارتفاع نسبة وجود مشاكل في أمراض القلب والأوعية الدموية.

الكلمات المفتاحية: فشل الكلى المزمن، الإنترلوكين-6، اضطراب العظم والمعدني في فشل الكلى المزمن، أمراض القلب والأوعية الدموعية، كثافة العظام.