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College of Medicine
Department of chemistry and Biochemistry

Study the Effect of Long Term Use of Glucocorticoid on Bone and its Mechanism of Inducing Osteoporosis in Babylon Governorate

A Thesis

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By

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بسم الله الرحمن الرحيم

" وقل اعملوا فسيرى الله عملكم ورسوله والمؤمنون "

صدق الله العلي العظيم

سورة التوبة اية (105)

Supervisor Certification

We certify that this thesis was prepared under our supervision at the College of Medicine /University of Babylon, as a partial fulfillment for the requirement for the degree of Master in Clinical Bio Chemistry

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Dedication

To my family, who always picked me up on time and encouraged me to go on every adventure, especially this one

Ayat J. K.

Acknowledgment

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Summary

Glucocorticoids are one of those medications that are used in treating various conditions, such as allergy, asthma, arthritis, and cancer metastasis. With the long-term usage of glucocorticoids, especially long-term high-dose applications, a series of adverse effects would appear. These include osteoporosis, hyperglycemia, insulin resistance, hypertension, severe infection, Cushing-like syndrome, peptic ulcers, and neuropsychiatric disorders. Glucocorticoids are the most common cause of secondary osteoporosis, so-called glucocorticoid-induced osteoporosis. The essential actors in effect of long term use of glucocorticoid of inducing osteoporosis include parathyroid hormone (PTH), calcium, and klotho.

The study's objective is to investigate the relationship between serum levels of PTH, klotho, and calcium in patients with long term use of glucocorticoid osteoporosis and healthy controls. This study was designed as a case-control study. 50 patients were involved in this study (31 females and 19 males) in addition to 50 controls (31 females and 19 males) well-matched with the patients in age and sex.

Body mass index (BMI) with (24-27)Kg/m^2 for patients and age was (20- 50) year mean age (39.31 ± 2.07) BMI with (26.96 ± 0.8) kg/m^2 for control Patients. Samples have been taken from clinics at Marjan Teaching Hospital, Imam Al-Sadiq Hospital, and Al-Hilla Teaching Hospital.

The levels of PTH, klotho were measured by ELISA Technique whereas the level of calcium was measured by colorimetric method according to the manufacturer manual .As the results of the tests that were conducted showed that the levels of calcium with long term use of inducing osteoporosis were significantly lower than healthy people, as the value of P <0.05. The levels of PTH and klotho in patients were

significantly higher than healthy people, as the value of P <0.05. Relationship showed significant correlation between klotho and calcium in long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients. There was a significant correlation between klotho and PTH (P≤0.01). Also significant correlation between klotho and calcium (p<0.001) within long term use of glucocorticoid patients. The receiver operating characteristic curve test showed a good discriminative value of klotho, and PTH between long term use of glucocorticoid in patients and controls. However, the recerver operating characteristic curve test showed a good discriminative value of klotho and PTH between long term use of glucocorticoid in patients and controls. The current study that we conducted leads us to the conclusion that klotho and PTH could be served as a prospective marker in long term use of glucocorticoid patients to predict the possibility to develop osteoporosis at long term use of glucocorticoids.

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List of Abbreviations

Abbreviations	Meaning
11β-HSDs	11β-hydroxysteroid dehydrogenases
AP-1	activator protein 1
ALP	alkaline phosphatase
ARE	antioxidant responsive element
AUC	area under curve
AIRE	autoimmune regulatory gene
Bcl2	B-cell lymphoma 2
BMI	Body mass index
BMD	Bone mineral density
BMP	bone morphogenetic protein
CaSR	Calcium-sensing receptors
JNK	c-Jun N-terminal kinase
CTX	C-terminal telopeptides of type I collagen
DEX	Dexamethasone
DKK1	Dickkopf-1
DNMT3a	DNA methyltransferase 3 alpha
DXA	dual-energy x-ray absorptiometry
ECG	Electrocardiograph
ELISA	enzyme-linked immunosorbent assays
FGF	fibroblast growth factors
FGF 19	Fibroblast growth factors19
FGF 21	Fibroblast growth factors21
FGF 23	Fibroblast growth factors23
FDA	Food and Drug Administration
FRAX	Fracture Risk Assessment Tool
GI	Gastrointestinal
GIO	Glucocorticoid induce Osteoporosis

GR	Glucocorticoid Recepter
GCs	Glucocorticoids
HSL	hormone-sensitive lipase
HRP	Horseradish Peroxidase
IJO	Idiopathic juvenile Osteoporosis
IGFs	Insulin growth factors
Wnt	int and Wg" Wingless-related integration site".
IL-11	Interleukin-11
IL-2	interleukin-2
KDa	Kilo Dalton
Kl1	Klotho1
Kl2	Klotho2
MC3T3-E1	Mouse Calvaria3T3-E1
Nrf-2	nuclear factor erythroid 2–related factor 2
NF-κB	nuclear factor kappa B
NF-kB	nuclear factor kappaB
OCN	Osteocalcin
P-val	p value
PTH	Parathyroid hormone
PTHrP	parathyroid hormone-related protein
PI3K/Akt/mTOR	phosphatidylinositiol 3-kinase /protein kinase B /mammalian Target of Rapamyci
PEPCK	Phosphoenolpyruvatecarboxykinas
Pyk2	Protein-tyrosine kinase 2 beta
PPIs	proton pump inhibitors
REM	Rapid Eye Movement
ROS	reactive oxygen species
ROC	Receiver operating characteristic

RANKL/OPG	Receptor activator of nuclear factor kappa-B ligand /Osteoprotegerin
RANK	receptor activator of nuclear factor-kappa B
RUNX2	runt-related transcription factor2
SHP	secondary hyperparathyroidism
SN	Sensitivity
SNP Rs9536314	Single Nucleotide Polymorphisms of Klotho
SGLT2	sodium-glucose cotransporter-2
SP	Specificity
SD	Standard deviation
SE	Standard error
TBS	trabecular bone score
TGFs	transforming growth factors
TNFα	tumor necrosis factor-Alpha
WBC	white blood cell

1. Introduction

1.1 Glucocorticoids

Synthetic glucocorticoids (GCs), drugs that are structurally and pharmacologically similar to the endogenous hormone cortisol, are used to treat a wide range of diseases, most often chronic diseases such as rheumatologic disorders, autoimmune diseases, allergies, or respiratory diseases[1].

Glucocorticoids possess various anti-inflammatory, immunosuppressive, metabolic, and endocrine properties, which have been hypothesized to be potentially either harmful or beneficial regarding diseases development[2].

The long-term use of glucocorticoids in patients with chronic inflammatory diseases, such as rheumatoid arthritis, is associated with severe long-term adverse effects, such as cardiovascular disorders, infections, and osteoporosis[3].

Glucocorticoids are one of those medications that are regularly prescribed in both inpatient and outpatient departments. Their anti-inflammatory activity is generally used in treating various conditions, such as allergy, asthma, arthritis, inflammatory bowel disease, and cancer metastasis. However, they have numerous side effects, such as fluid retention, weight gain, and hyperglycemia. With the long-term usage of glucocorticoids, especially long-term high-dose applications, a series of adverse events would appear. These adverse events include osteoporosis, hyperglycemia, insulin resistance, hypertension, muscle atrophy, severe infection, Cushing-like syndrome, peptic ulcers, and neuropsychiatric disorders. Actually, it is a real practical skill of a doctor to be able to properly use glucocorticoids[4].

Cortisone (hydrocortisone) is physiologically similar to the internal hormone, and mainly used in the alternative treatment of adrenal insufficiency and other endocrine diseases. In critical conditions, such as sepsis, severe asthma, severe drug rashes, and acute nephritis, high doses of glucocorticoids are used intravenously. Glucocorticoids could not be used as simple antipyretic analgesic drugs. Glucocorticoids should be given locally when patients present with local inflammation, i.e., intra-articular injection is used for the treatment of arthritis, inhalation for asthma, and local application for dermatitis. The anti-inflammatory activities of classical glucocorticoids are useful for short-term use in most patients, but their chronic and systemic use usually causes side effects, and results in reducing sensitivity. The adverse effects of glucocorticoids are usually associated with high dose and long duration usage.the treatment with glucocorticoids for more than 3 months leads to a 7-fold increase in hip fractures and a 17-fold increase in vertebral fractures. Glucocorticoids are the most common cause of secondary osteoporosis, so-called glucocorticoid-induced osteoporosis[5].

1.1.1 Structure and Function of Glucocorticoids

Although glucocorticoids have different names, they have similar biological effects. The commonly used glucocorticoids are prednisone, prednisolone, triamcinolone, methylprednisolone, dexamethasone, and others. They are modified in the chemical structures of the cortisone (hydrocortisone) backbone in order to enhance their anti-inflammatory effects. The chemical modifications include introduction of 6α-fluoro substitution, and reducing the binding to the mineralocorticoid receptor by insertion of a C=C double bond at C1, C2, or replacing a lipophilic substituent such as

21α-esters attached to the D-ring for increasing binding to glucocorticoid receptors figure.1-1[6].

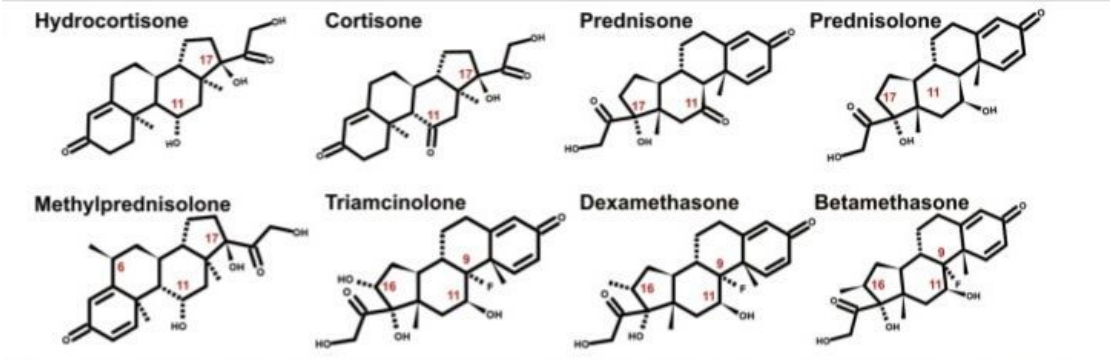


Figure 1-1. The chemical structure of common glucocorticoids[6].

1.1.1.1 Metabolic Functions

Glucocorticoids acts on glucose metabolism to cause hyperglycemia. This effect is not only involved in maintaining normal glucose homeostasis but at times of stress, glucose happens to be the only substrate that provides energy to the critical organs of the body such as the brain and skeletal muscles during times of stress such as an illness or exercise. Hyperglycemia is caused by increasing the synthesis of enzymes involved in glycogenolysis and gluconeogenesis[7].

Glucocorticoids upregulates or activates or induces enzymes involved in gluconeogenesis and glycogenolysis. It antagonizes the actions of insulin and decreases the cellular uptake of glucose to increase the availability of glucose for the brain, red blood cells, and skeletal muscles. GCs increases gluconeogenesis by inducing the gene expression of the Phosphoenolpyruvate carboxykinase (PEPCK) enzyme. This step occurs in the cytosol; fructose-1,6-bisphosphate converts to fructose-6-phosphate. By antagonizing the actions of

insulin, it decreases (a) glycogen synthesis and (b) glucose uptake by glut four transporters[8].

Glucocorticoids(GCs), to further increase the gluconeogenetic substrates, establish a catabolic state in muscles, inducing peripheral muscle breakdown and mobilizing amino acids towards the liver to be used in gluconeogenesis (formation of glucose from amino acids). Furthermore, glucocorticoids activate hormone-sensitive lipase (HSL) in the adipose tissue resulting in increased availability of free fatty acids for beta-oxidation. These metabolic actions of glucocorticoids explain many of the effects of exogenous glucocorticoid medication. Glucocorticoids result in decreased muscle mass; skin gets thinner, fragile, and easy to bruise. Glucocorticoids also result in hyperglycemia and lipodystrophy (redistribution of fat in the back of the neck- buffalo hump, face -moon face- and decrease of adipose tissue in extremities). The mechanism of this fat redistribution is unknown figure.1-2 [9].

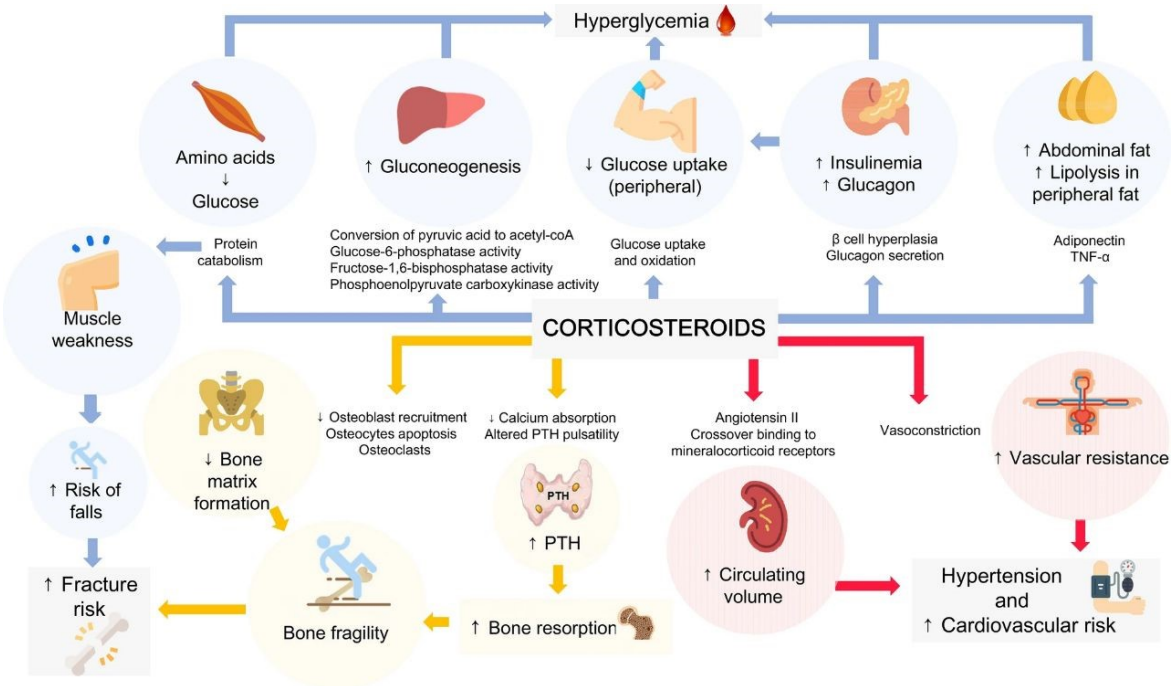


Figure 1-2. The Metabolic Functions of glucocorticoids[10].

1.1.1.2 Anti-inflammatory and Immune-Suppressive Function

Glucocorticoids result in a net increase in the white blood cell(WBC) count. The increased WBC count, is a combination of a decrease in neutrophil migration in tissues, an inhibition of neutrophil apoptosis, and promote WBC maturation in the bone marrow, and release in circulation. With regards to eosinophils, glucocorticoids induce apoptosis and sequestration of eosinophils in the periphery. Inhibition of interleukin-2 (IL-2) signaling (inhibition of T cell proliferation), the impaired release of cells from lymphoid tissues, T lymphocyte apoptosis, inhibition of NF-kB (decrease in cytokine gene expression) and degranulation inhibition of mast cells are effects of glucocorticoids in lymphatic tissue. In the setting of increased glucocorticoid levels in the blood, the macrophages of the reticuloendothelial system fail to recognize and phagocytose antigens (even opsonized antigens). A sequela of these effects is the regression in size of lymphoid tissue (thymus, spleen and lymph nodes) figure.1-3 [11].

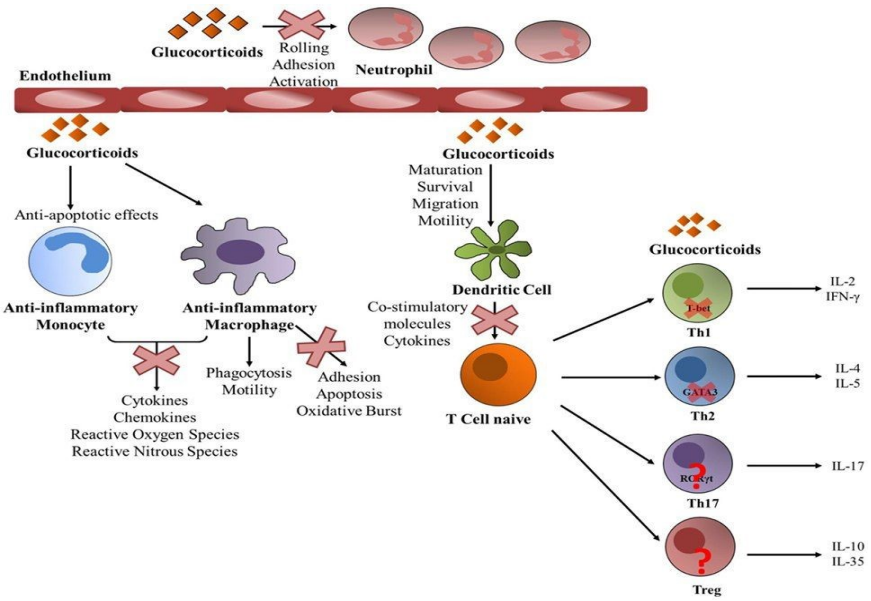


Figure 1-3. Anti-inflammatory and Immune-suppressive Function of glucocorticoids[12].

1.1.1.3 Neurologic Effects

Several individuals who present with hypercortisolism (by receiving exogenous doses of glucocorticoids or suffer from Cushing syndrome) may present with depression. This effect of hypercortisolemia, a possible result of glucocorticoid-induced neuronal excitation, may have a role in the pathogenesis of the major depressive disorder. Affected hypercortisolemic patients present with difficulty falling a sleep, a decrease in Rapid eye movement (REM) sleep latency, and slow-wave sleep. Also, alterations in electroencephalogram patterns are frequent in these individuals. Chronic exogenous glucocorticoid administration has such detrimental effects. Extended glucocorticoid use can cause complications such as cardiovascular disorders, infections, and osteoporosis [13].

1.1.2 Long Term Effect of High Level of Glucocorticoids

The long-term use of glucocorticoids in patients with chronic inflammatory diseases, such as rheumatoid arthritis, is associated with severe long-term adverse effects, such as cardiovascular disorders, infections, and osteoporosis. Glucocorticoids such as cortisone are very effective in controlling inflammatory diseases. However, this type of drug suppresses the adrenal glands, which impairs the body's ability to produce its own cortisone, according to the study. This can cause fatigue, low blood pressure, and nausea, and can potentially be life-threatening in some cases figure. 1-4[14].

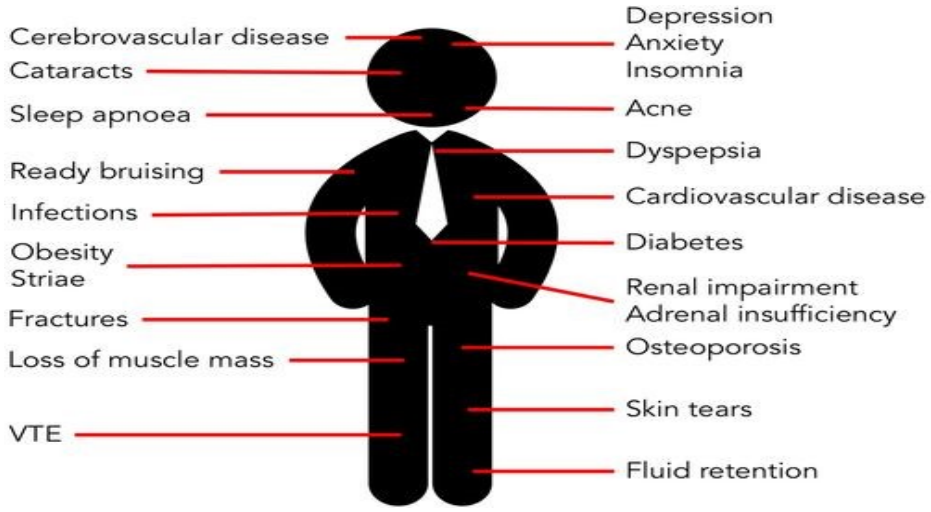


Figure 1-4. Long term effect of high level of Glucocorticoids [15].

1.1.3 The mechainism of Glucocorticoids on the Bone structure causing Osteoporosis.

1.1.3.1 Direct Action of Glucocorticoid on Osteoblasts

Glucocorticoid-induced osteoblast dysfunction is the main pathological mechanisms underlying the development of Glucocorticoids Induced Osteoporosis . GCs impair osteoblast proliferation, increase apoptosis and alter autophagy through changing RANKL/OPG and Wnt/sclerostin expression. Their inhibitors, microRNAs, IL-11, BMP/notch signalling, and effectors of apoptosis also play a major role in the action of GCs on the osteoblasts. GCs suppress the stimulates osteoblast differentiation and activity, partially through the enhancement of the DKK1 production in cultured human osteoblasts . Using dexamethasone (DEX) as an example, at higher concentrations, it inhibits osteoblast differentiation by decreasing alkaline phosphatase (ALP) activity, RUNX2 and osteocalcin (OCN) expressions, and increasing RANK expression. The actions of GCs on bone are also determined by variations in the expression and

sensitivity of the GC receptors, the export of steroids from the cell by transmembrane transporters, and enzymatic metabolism of GCs to the more or less active metabolites [16].

The compound 11β-hydroxysteroid dehydrogenases (11β-HSDs), which control the interconversion between the active cortisol and corticosterone and their inactive counterparts, cortisone and dehydrocorticosterone, also contribute to the skeletal action of GC on bone. All these events contribute to increased bone turnover, reduced mineralization, and subsequently, bone loss. GCs are important in the differentiation of osteoblasts, but their effects on osteoblast proliferation are inconsistent. At lower doses (10^{-7}M DEX), GCs promote uncommitted mesenchymal precursors cells to differentiate into osteoblasts. However, at high doses (>10^{-7}M DEX), GCs inhibit the proliferation of osteoblast-like cells in culture . GCs decrease bone formation in most in vivo studies but stimulate it in in vitro studies. These opposing effects could be due to low levels of GCs being stimulatory at lower concentrations and inhibitory at higher concentrations.Multipotent mesenchymal precursor cells could develop into osteoblasts, adipocytes or other cell lineages. GCs facilitate the commitment of these precursors cells to differentiate into certain cell types [17,18].

Glucocorticoids stimulate the expression of markers of differentiation of both osteoblasts and adipocytes in in vitro studies. In the human studies, GCs are associated with bone marrow adiposity within the femoral neck [19].

Genetic deletion of 11β-HSD1 and the lowering of GC levels were reported to affect marrow adipose tissue but not bone formation. GCs upregulate DKK1 expressed by the osteoblasts, which in turn

suppress anabolic osteoblast behavior. GCs also stimulate osteoblast apoptosis *in vitro* via the increased endoplasmic reticulum stress through a synergistic pathway with TNFα. This triggers the rapid activation of the kinases Pyk2 and JNK and increases reactive oxygen species in primary cultures. The Bcl2 family such as Bim is the proapoptotic factor, and its expression is regulated by GCs. GCs also upregulate Bak expression and downregulate Bcl-xL expression (prosurvival). DEX can also induce Bcl2-mediated cell death via the induction of p53. Insulin growth factors (IGFs), transforming growth factors (TGFs), fibroblast growth factors (FGF), and platelet-derived growth factors are also other signalling pathways also targeted by GCs. GCs reduce the anabolic actions of TGFβ and suppress the expression of IGF-I and platelet-derived growth factors, which possess anabolic mitogenic actions in osteoblasts figure.1-5[20].

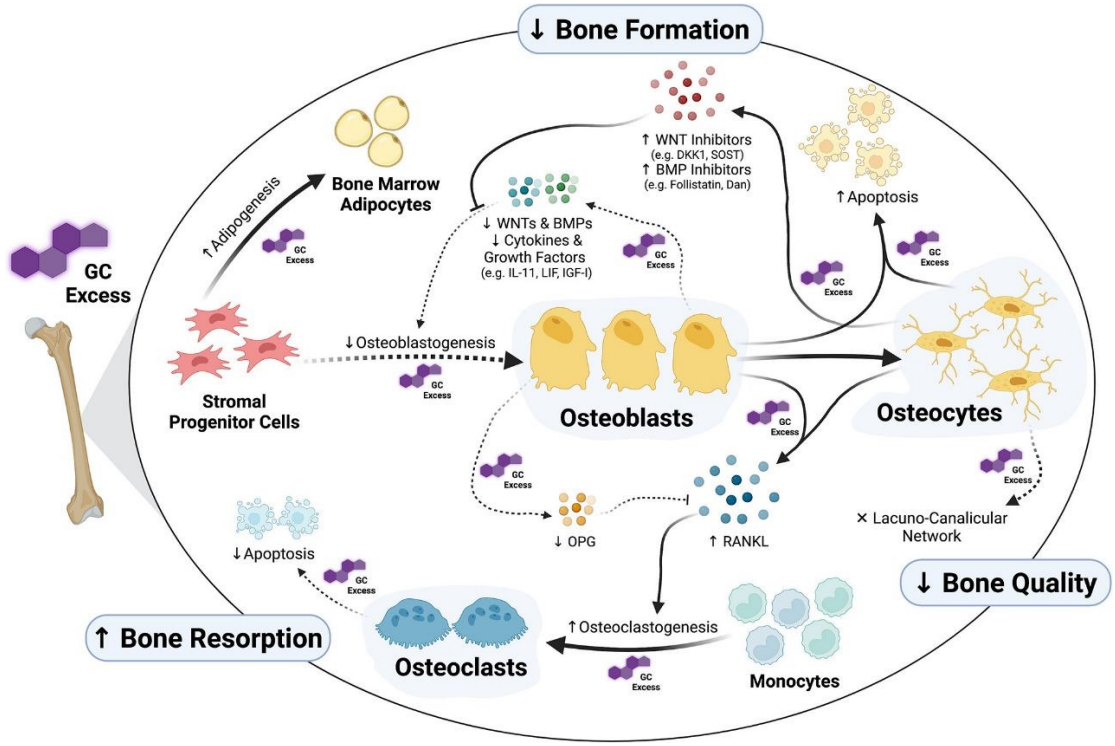


Figure 1-5. Osteoblasts and osteocytes as main targets of glucocorticoid (GC) excess in the skeleton[21].

The suppression of IL-11 has been identified as an important mediator of the adverse effects GCs on bone. Therapeutic GCs reduce sex steroid levels which is likely to impact patients with serious inflammatory illnesses greater due to the effects of inflammation on the hypothalamic–pituitary–gonadal axis. This cascade will be harmful to the bone . GC excess leads to central fat accumulation due to its impacts on fat metabolism, which is associated with increased insulin resistance. Obesity and diabetes both have complex impacts on bone metabolism and fracture risk [22,23].

1.1.3.2 Glucocorticoid and Oxidative Stress in Osteoblasts

Glucocorticoids can cause oxidative stress through the production of reactive oxygen species (ROS), downregulation of cytoprotective antioxidant proteins and antioxidant enzyme activities. High ROS levels inhibit osteoblast differentiation and function, causing osteoblast cell death and growth reduction [24].

DEX treatment induces oxidative damage by depleting total antioxidant capacity while increasing ROS formation and lipid peroxidation. It also causes a significant reduction in the RUNX2 mRNA expression, which underlies high-dose DEX-induced osteotoxicity. DEX treatment also triggers a significant decline in the mitochondrial membrane potential due to upregulated caspase activity. Treatment with antioxidants can upregulate the expression levels of these osteogenic markers and downregulate caspase expression, thus decreasing the apoptotic effect of DEX. This observation suggests the involvement of oxidative stress in DEX-induced osteoporosis. The study found that DEX-treated MC3T3-E1 pre-osteoblast cells express lower nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) and their target proteins with a decline in oxidative stress markers. This negative impact could be reversed with plumbagin, which is an antioxidant. Nrf-2 is a transcriptional activator, which binds to antioxidant responsive element (ARE) and enhances the expression of antioxidant enzymes. This endogenous antioxidant defence is activated in a cellular oxidative stress event. ROS also induces endoplasmic reticulum stress and autophagy-mediated apoptosis. The study demonstrated that DEX induces apoptosis, endoplasmic reticulum stress, ROS formation and autophagy in pre-osteoblasts [25].

The studies demonstrated that high-dose DEX treatment reduced bone generation and destroyed bone trabeculae, leading to microarchitectural degenerative changes mimicking osteoporosis [26,27].

Therefore, inhibiting the production of ROS may provide an avenue of intervention against GC-induced apoptosis of MC3T3-E1 cells. Most in the studies showed that GCs usually cause a decrease in bone formation, but some in vitro studies showed largely stimulatory effects of GCs actions. This observation may suggest that a low GC level possesses stimulatory effects, while a high GC level possesses inhibitory effects on bone [28].

Glucocorticoids exert anti-inflammatory effects on osteoblasts by suppressing cytokines, such as IL-11, via the interaction of the monomeric GR with AP-1 but not nuclear factor kappa B (NF-κB). The inhibition of cytokines by GCs attenuates osteoblast differentiation, which partly accounts for bone loss during GC therapy [29].

1.1.3.3 Action of Glucocorticoid on Osteoclasts

Glucocorticoids create an environment favouring osteoclast formation and bone resorption activities by increasing RANKL and suppressing OPG secretion by osteoblasts [30]. This process may be mediated by miR-17/20a in osteoblasts and miR-182 in osteoclasts. GCs also affect osteoclast functions directly. GCs can induce osteoclast-mediated bone resorption without affecting their apoptosis rate, and this process requires the dimeric GC receptor[31].

Glucocorticoids can improve autophagy in osteoclasts and promote their survival through the PI3K/Akt/mTOR signalling

pathway. GCs can affect the geometry of osteoclast resorption activities by forming more trench-like resorption pits, which directly affect bone stiffness, with the lumbar as the most affected bone site [32].

At a similar exposure level, GCs can induce mitochondria dysfunction and oxidative stress in osteoblasts but not osteoclasts; this may contribute to the imbalanced bone remodelling observed in GIO. However, prolonged GC exposure may be destructive to osteoclasts and their functions, hindering bone remodelling cycle and predisposing users to osteoporosis [33].

1.2 Osteoporosis

Osteoporosis is defined as low bone mineral density caused by altered bone microstructure, ultimately predisposing patients to low-impact, fragility fractures. Osteoporotic fractures lead to a significant decrease in quality of life, increasing morbidity, mortality. [34].

Over 50% of postmenopausal white women will have an osteoporotsis-related fracture. Only 33% of senior women who have a hip fracture will be able to return to independence. In white men, the risk of an osteoporotic fracture is 20%, but the one-year mortality in men who have a hip fracture is twice that of women. Black males and females have less osteoporosis than their white counterparts, but those diagnosed with osteoporosis have similar fracture risks figure.1-6 [35][36].

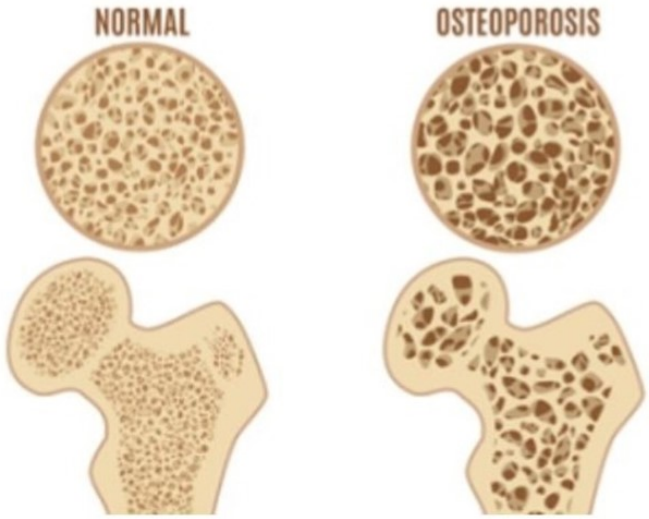


Figure 1-6. Difference between normal Bone Shape and Osteoporosis[37].

1.2.1 Types of Osteoporosis

1.2.1.1 Primary Osteoporosis

This is the most common type of osteoporosis and occurs more in women than men. Primary osteoporosis is usually caused by age-related factors, and may be referred to as senile osteoporosis, or when the cause is unknown, idiopathic osteoporosis[38].

Peak bone density (mass) is reached between the ages of 25 and 30 yrs, bone loss slowly begins to increase. With increased bone loss, the rate of bone generation will also decrease. The chances of developing osteoporosis depend on the density of one's bones earlier in life. Diet, health and physical exercise will also determine bone density (to a degree) throughout life[39].

Accelerated loss of bone density will usually begin after a women's monthly menstrual cycle comes to an end (i.e. during menopause) which occurs when the production of oestrogen begins to slow down (this is usually around the age of 45 to 55).In men, gradual bone loss will normally begin between the ages of 45 and 50, this is when testosterone production begins to slow.Osteoporosis usually only impacts people when they are over the age of 60[40].

1.2.1.2 Secondary Osteoporosis

This type of osteoporosis has similar symptoms to those commonly seen in primary osteoporosis, however, secondary osteoporosis occurs as a result of certain medical conditions such as leukaemia, hyperthyroidism or hyperparathyroidism[41].

This type of osteoporosis may also result from taking certain medications that lead to the breakdown of bones, these include high-dose inhaled or oral corticosteroids that have been used. Other medications include high doses of thyroid hormone replacements or drugs known as aromatase inhibitors which are used in the treatment of breast cancer.Secondary osteoporosis can affect any age[42].

1.2.2 Drug-induced Osteoporosis

Drug-induced osteoporosis is the second most common cause of secondary osteoporosis. Despite their well-known adverse events, glucocorticoids are still one of the cornerstone immune-suppressive/modulator and anti-inflammatory therapies. Up to 40% of patients on long-term glucocorticoid therapy suffer from fractures during their lifetime. Areas with high trabecular bone, such as lumbar spine and hip trochanter, are the classic sites for glucocorticoid-induced fractures .The fracture risk with glucocorticoid therapy is dose and time-dependent. The impact of glucocorticoids on bone has been linked to their cumulative effect, which disturbs both bone quantity and quality. Glucocorticoids can induce bone loss irrespective of the route of administration. For instance, long-term inhaled glucocorticoids were associated with a 10% loss of BMD [43].

Even controlled-release and topical corticosteroid can negatively impact bone health [44].

Glucocorticoids initially decrease bone formation and increase RANKL/osteoprotegerin ratio, inducing high bone resorption.The mechanism of bone loss with long-term usage is more attributed to suppressed bone formation rather than increased bone resorption. This could be due to the downregulation of the Wnt (wingless-related integration site) signaling pathway which impairs the osteoblast activity. Additionally, glucocorticoids have an indirect impact on bone through their effects on calcium homeostasis, parathyroid gland activities, and vitamin D metabolism. Furthermore, glucocorticoids lead to loss of muscle mass and strength which increases the risk of falls and fractures. They can also induce hypogonadism which

decreases the anti-resorptive effect of testosterone and/or estrogen [45].

The use of a DXA scan and FRAX after 6 months of glucocorticoid therapy is recommended for those with a history of fragility fracture, patients of 40 years of age or older, and those with major osteoporotic risk factors. For prevention of glucocorticoid-induced osteoporosis, daily intake of 1000–1200 mg calcium and 600–800 units of vitamin D, along with lifestyle modification, are highly recommended. For adults with high risk of fracture, treatment with oral bisphosphonate is the preferred line of therapy. Teriparatide is also effective in preventing and treating glucocorticoid-induced bone loss. Antidepressants like selective serotonin reuptake inhibitors and monoamine oxidase inhibitors can induce low bone density and increase incidence of fracture figure. 1-7 [46,47].

Figure 1-7. DEXA Scanner[48].

It is not clear how these medications affect bone health, but it may be attributed to diminished osteoblast proliferation through the

serotonin receptors and transporters. Many studies showed significant bone loss with long-term use of antiepileptic drugs [49].

Antidiabetic medications can impact bone health either positively or negatively. Peroxisome proliferator-activated receptor gamma (PPARγ) plays an important role in the regulation of bone formation and energy metabolism, along with insulin sensitivity [49]. Its stimulation by thiazolidinediones induces bone resorption and inhibits bone formation. Thiazolidinediones decreased the BMD and increased the risk of osteoporosis when compared to other anti-diabetic medications [50].

The effects of sodium-glucose cotransporter-2 (SGLT2) inhibitors on bone metabolism and fracture risk are receiving more attention because of their wide use. They may increase bone turnover, disturb bone microarchitecture, and reduce BMD. In 2010, the FDA released a warning against long-term use of proton pump inhibitors (PPIs) as it may increase the incidence of osteoporosis and fracture risk [51][52].

The limited available evidence suggested that this might happen through histamine over-secretion, and affecting mineral homeostasis. There is inconsistent data regarding the impact of PPIs on BMD.

Despite the negative effect of anticoagulants on bone metabolism having been studied for a long time, such effect is still debatable, and the underlying mechanisms are still poorly understood [53].

Unfractionated heparin was associated with significant bone loss compared to low molecular weight heparin[54,55 ,56]. Long-term use of warfarin was associated with decreased BMD and TBS [57]. In a recent study, this negative effect on bone was more pronounced in warfarin but was also found in direct oral anticoagulants [58].

1.3 Parathyroid Hormone

Parathyroid hormone(PTH) is a polypeptide that is synthesized and cleaved into an active form within the parathyroid gland. The initial structure formed is a pre-pro-PTH, a 115 amino acid polypeptide that is cleaved to form pro-PTH comprised of 90 amino acids. It is then cleaved a second time, again at the amino-terminal portion, to form active parathyroid hormone comprised of 84 amino acids. This is the primary hormone that is stored, secreted, and functions in the body. The process of synthesis, cleavage, and storage is estimated to take less than an hour. Active PTH secretion can occur as quickly as a few seconds when low serum calcium is detected. The mechanism of secretion is via exocytosis, a process where the hormone is released through a membrane vesicle carried to the cell membrane, releasing the hormone after the vesicle fuses with the outer membrane. The serum half-life of activated PTH is a few minutes and is removed from the serum quickly by the kidney and liver(figure.1-8 [59][60]).

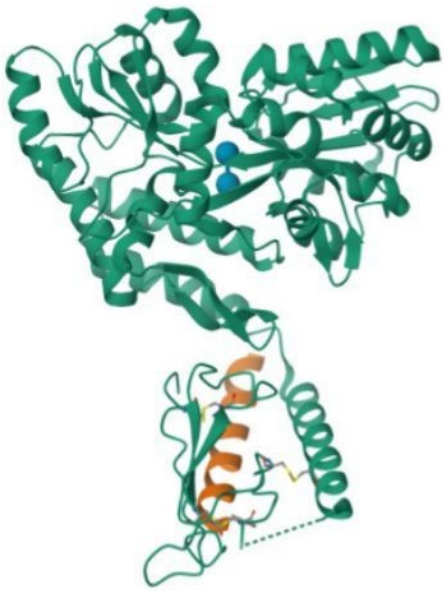


Figure 1-8. Crystal Dimer Structure of PTH [61]

In the blood, the sensitive process of calcium and phosphate homeostasis is maintained primarily by an appropriately functioning parathyroid gland. The parathyroid gland is comprised of 4 small glands located posteriorly to the thyroid in the middle aspect of the anterior neck. The parathyroid gland secretes parathyroid hormone (PTH), a polypeptide, in response to low calcium levels detected in the blood. PTH facilitates the synthesis of active vitamin D and calcitriol (1,25-dihydroxycholecalciferol) in the kidneys. In conjunction with calcitriol, PTH regulates calcium and phosphate. PTH effects are present in the bones, kidneys, and small intestines. As serum calcium levels drop, the secretion of PTH by the parathyroid gland increases. Increased calcium levels in the serum serve as a negative-feedback loop signaling the parathyroid glands to stop the release of PTH. The mechanism of PTH in the body is intricate, and the clinical ramifications of irregularities are significant. The understanding of PTH is of paramount relevance and importance figure.1-9[62][63][64][65].

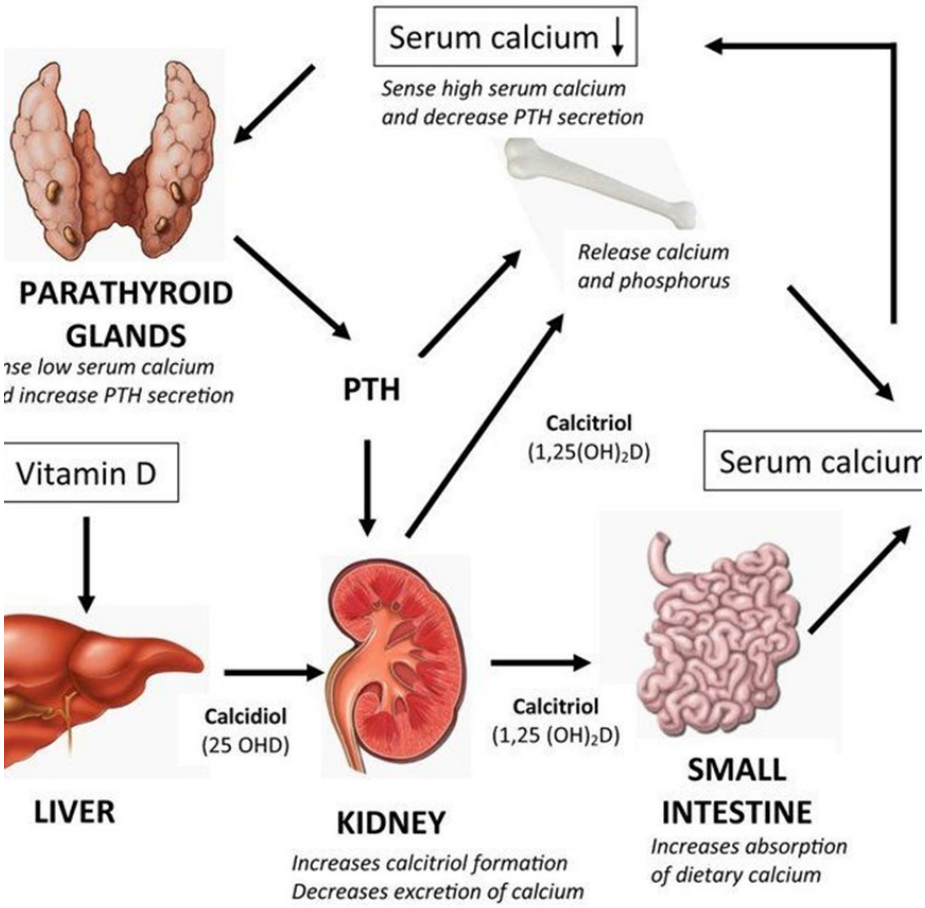


Figure 1-9. Sensitive process of calcium homeostasis by Parathyroid hormone[66].

1.3.1 Effects of PTH in the Bones, Kidneys, and Small Intestines.

In the bones, PTH stimulates the release of calcium in an indirect process through osteoclasts which ultimately leads to resorption of the bones. However, before osteoclast activity, PTH directly stimulates osteoblasts which increases their expression of Receptor activator of nuclear factor kappa-B ligand (RANKL), a receptor activator for nuclear factor kappa-B ligand, allowing for the differentiation of osteoblasts into osteocytes. PTH also inhibits the secretion of osteoprotegerin, allowing for preferential differentiation into osteoclasts. Osteoprotegerin normally competitively binds with RANKL diminishing the ability to form osteoclasts. Osteoclasts

possess the ability to remodel the bones (resorption) by dissolution and degradation of hydroxyapatite and other organic material, releasing calcium into the blood. In the kidneys, parathyroid hormone has 3 functions in increasing serum calcium levels. Most of the physiologic calcium reabsorption in the nephron takes place in the proximal convoluted tubule and additionally at the ascending loop of Henle. Circulating parathyroid hormone targets the distal convoluted tubule and collecting duct, directly increasing calcium reabsorption. Parathyroid hormone decreases phosphate reabsorption at the proximal convoluted tubule. Phosphate ions in the serum form salts with calcium that are insoluble, resulting in decreased plasma calcium. The reduction of phosphate ions, therefore, results in more ionized calcium in the blood[67].

Starting at the kidneys, PTH stimulates the production of 1alpha-hydroxylase in the proximal convoluted tubule. This enzyme, 1alpha-hydroxylase, is required to catalyze the synthesis of active vitamin D - 1,25-dihydroxycholecalciferol from the inactive form 25-hydroxycholecalciferol. Active vitamin D plays a role in calcium reabsorption in the distal convoluted tubule via calbindin-D, a cytosolic vitamin D-dependent calcium-binding protein. In the small intestine, vitamin D allows the absorption of calcium through an active transcellular pathway and a passive paracellular pathway. The transcellular pathway requires energy, while the paracellular pathway allows for the passage of calcium through tight junctions[68].

1.3.2 Pathophysiology

The two umbrella categorizations of parathyroid dysfunctions are hyperparathyroidism and hypoparathyroidism. The inappropriately high secretion of PTH is classified as hyperparathyroidism, while the inappropriately low secretion of PTH is designated as hyperparathyroidism[69].

1.3.2.1 Hyperparathyroidism

Hyperparathyroidism is further characterized as primary, secondary, and tertiary dysfunction. Primary hyperparathyroidism refers to an abnormality to the parathyroid gland itself, such as an adenoma or hyperplasia causing the gland to oversecrete. This is characterized by lab values that show elevated PTH levels, hypercalcemia, and hypophosphatemia. Primary hyperparathyroidism is customarily due to an adenoma, hyperplasia, or even more rare, a carcinoma. Adenomas are very sporadic and can be surgically resected. Hyperplasia can be found in cases of multiple endocrine neoplasia (MEN) types I and IIa and in an autosomal dominant condition called familial hypocalciuric hypercalcemia. In MEN type I, patients are often characterized by having tumors in the pituitary gland, parathyroid gland, and pancreas[70].

MEN type IIa is characterized by the presence of medullary thyroid carcinoma, pheochromocytoma, and parathyroid hyperplasia. In familial hypocalciuric hypercalcemia, there is a mutation of the calcium-sensing receptor in the parathyroid gland and kidney, resulting in a higher-than-normal setpoint. This causes a lack of inhibition of PTH secretion until a higher level of serum calcium, thus resulting in increased bone resorption and hypercalcemia.

Hypercalcemia is further exacerbated with increased renal absorption of calcium, resulting in hypocalciuria. These conditions are rare and are not always favored for surgical resection. Patients with hyperparathyroidism will have correlated hypercalcemia which can cause symptoms of excessive thirst and urination, constipation, bone pain, fatigue, depression, and possibly kidney stones. This is commonly memorized as "stones, bones, groans, thrones, and psychiatric overtone[71].

Secondary hyperparathyroidism refers to the compensatory oversecretion of PTH in response to abnormally low calcium in the blood due to other pathological processes such as renal failure, gastrointestinal malabsorption, or simply a vitamin D deficiency. Lab values differ according to the underlying pathology. In chronic renal failure, there will be elevated PTH, but with decreased calcium and elevated phosphate. In the setting of malabsorption and vitamin D deficiency, there will be elevated PTH but decreased calcium and phosphate[72].

Tertiary hyperparathyroidism is exceedingly rare but is seen in the context of continuous PTH secretion even after a secondary hyperparathyroidism precipitating condition is resolved. Lab values will show moderately elevated PTH, normal or elevated calcium, and decreased phosphate[73] [74] [75].

1.3.3 Clinical Significance of PTH

1.3.3.1 Calcium's Role

Calcium is a divalent cation essential to heart, kidney, bone, and nervous system functioning, making PTH's functioning crucial. Calcium plays an integral part in cardiac contractions. The contractility of the heart is predicated on the availability and role of calcium inside myocardial cells. When there is an excess amount of calcium within cardiac cells, contractility will increase, and similarly, when there is a lesser concentration of calcium within the cardiac cells, contraction will decrease. This can potentially lead to prolonged QT intervals seen on ECG. Extreme hypercalcemia's effect on the myocardium can be manifested in ECG changes, causing very short QT intervals. This could potentially precipitate the onset of fatal arrhythmias such as ventricular tachycardia or even ventricular fibrillation if gone unattended. Abnormal oversecretion of PTH is also the source of bone degradation, systemically releasing dangerous amounts of calcium into the blood. This can facilitate the premature transition into osteoporosis and increase the susceptibility to fractures[76] [77] [78] [79].

1.4. Calcium

Calcium is a metal element with the symbol Ca and atomic number 20 [80] [81].

Calcium is an essential element that is required in large quantities. The Ca^{2+} ion acts as an electrolyte and is vital for the health of the muscular, circulatory and digestive systems; indispensable for building bones. It supports the synthesis and function of blood cells. For example, it regulates muscle contraction, nerve conduction, and blood clotting. As a result, calcium levels inside and outside the cell are tightly regulated by the body. Calcium can play this role because the Ca^{2+} ion forms stable coordination complexes with many organic compounds, especially proteinsThey also form compounds with a wide range of soluble substances, enabling the formation of the Skeleton[82].

Calcium is a healthy bone mineral. About 99% of the calcium in the body is stored in the bones and teeth. It is the metal that makes it hard and strong. The remaining 1% is required for many activities that help keep the body functioning normally. Bones are constantly being remodeled every day, and calcium moves in and out. In children and adolescents, the body builds new bone faster than it breaks down old bone, so overall bone mass increases. This continues until about age 30, when new bone formation and old bone breakdown begin at about the same rate. In older adults, especially postmenopausal women, bone is broken down at a faster rate than it is being built. If calcium intake is too low, it may contribute to osteoporosis [83].

1.4.1 Biological and Pathological Role of Calcium

Calcium ions can be accumulated by proteins through the binding of carboxyl groups to glutamic acid or aspartic acid residues; through interaction with residues of phosphorylated serine, tyrosine or threonine; or by chelating by carboxylic amino acid residus [84] [85].

As an example of the wide range of solubility of calcium compounds, monocalcium phosphate is highly soluble in water, 85% of extracellular calcium is dicalcium phosphate with a solubility of 2.0 mM in an organic matrix is tri-calcium phosphate at 100 μM. [86].

1.4.1.1 Hormonal regulation of Bone Formation and Serum level of Ca

Parathyroid hormone and vitamin D promote bone formation by allowing and promoting deposition of calcium ions, which allows for rapid bone turnover without affecting bone mass or mineral content.When plasma calcium levels fall, cell surface receptors are activated and PTH secretion occurs. It then proceeds to stimulate the entry of calcium into the plasma pool by taking it from the target kidney, intestine, and bone cells, with the bone-forming action of parathyroid hormone counteracted by calcitonin, whose secretion increases with increasing plasma calcium level figure. 1-9[87].

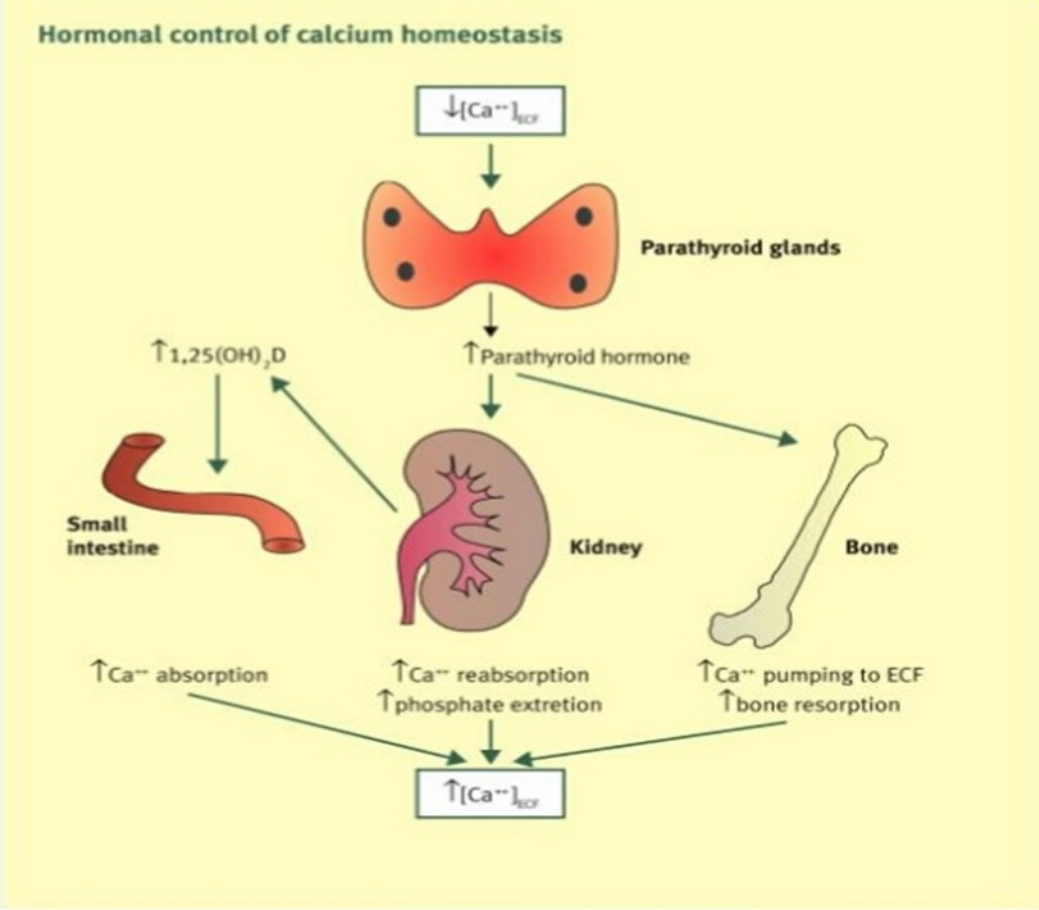


Figure 1-10. Hormonal regulation of Calcium Serum levels [88].

Excess calcium intake may lead to hypercalcemia. However, since the intestine absorbs calcium somewhat inefficiently, it is more likely that the cause of elevated serum calcium is excessive secretion of PTH or possibly due to excessive intake of vitamin D, both of which facilitate calcium absorption. All of these conditions lead to the deposition of excess calcium salts in the heart, blood vessels or kidneys. Symptoms include loss of appetite, nausea, vomiting, memory loss, confusion, muscle weakness, increased urination, dehydration, and metabolic bone disease[89].

Chronic hypercalcemia usually leads to calcification of soft tissues and its serious consequences: for example, calcification can cause a loss of elasticity of the walls of blood vessels and disruption of the

laminar blood flow;hence the rupture of plaques and thrombosis. Conversely, an insufficient intake of calcium or vitamin D may lead to hypocalcemia, which often also occurs due to insufficient secretion of PTH or PTH receptors in cells. Symptoms include neuromuscular excitability, which is likely to cause tetany and disruption of conduction in cardiac tissue[90].

Since calcium is essential for bone growth, many bone diseases can be traced back to organic matrix in the molecular structure or organization of bone. Osteoporosis is a decrease in bone mineral content per unit volume, and can be treated with calcium, vitamin D and bisphosphonate supplementation.Inadequate amounts of calcium, vitamin D or phosphate can lead to osteomalacia, which is called osteomalacia[91].

1.5 Klotho

Klotho is an enzyme that in humans is encoded by the KL gene; There are three klotho subfamilies: α-klotho, β-klotho, and γ-klotho.The word "klotho" often means the α-klotho sub-family, because α-klotho was discovered before other members of the sub-family [92].

α-klotho is main type and highly expressed in the brain , liver. Beta-klotho is predominantly expressed in the liver, skin. Klotho can exist in a membrane-bound form or in a soluble and circulating (hormonal) form. Proteases can convert the membrane bound shape to the round shape (figure. 1-10[93]) .

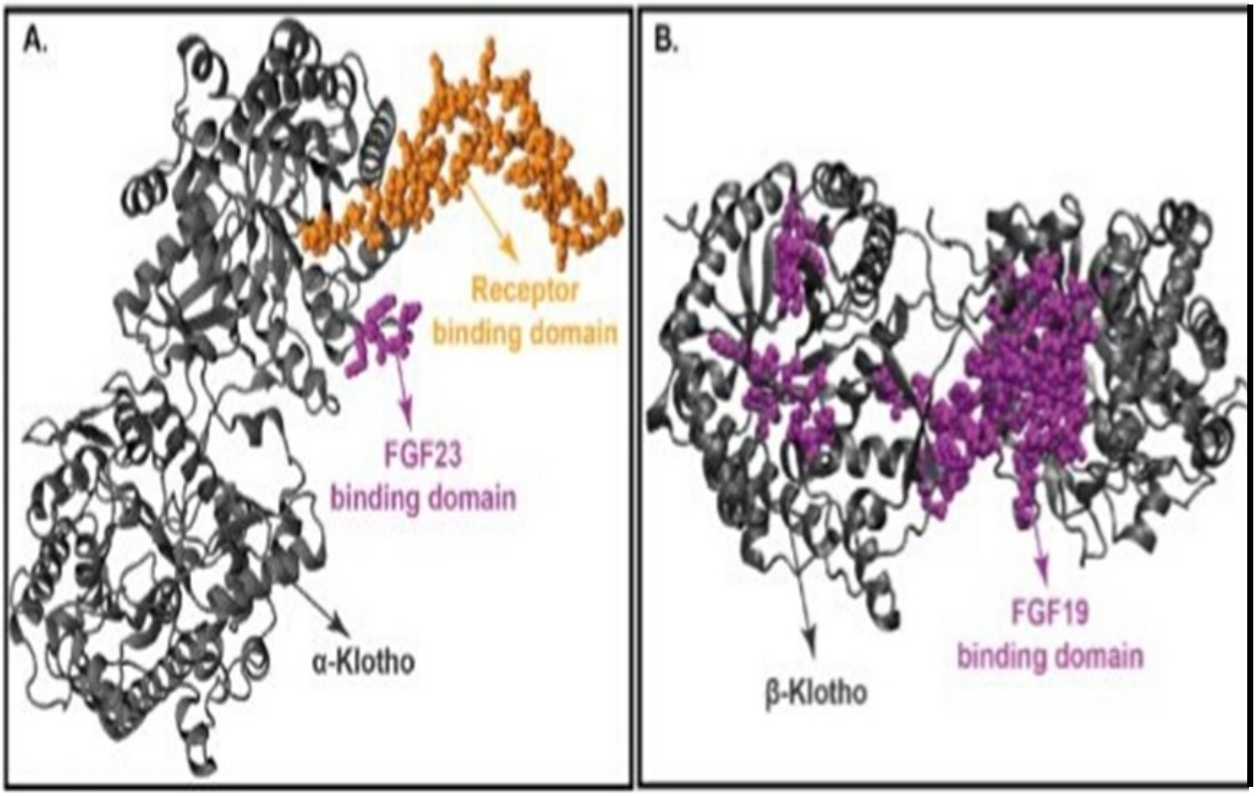


Figure 1-11. Crystal structure of klotho proteins. Panel-(A): Crystal structure of KLA. Panel-(B): Crystal structure of KLB. Both KLA and KLB's backbone is represented in grey, protein binding domain (FGF23 for KLA and FGF19 for KLB) shown in magenta, and receptor binding domain is represented in yellow[94] [95].

1.5.1 Klotho Functions

The α-klotho gene is located on chromosome 13, and is translated into a single-passed integral membrane protein. The intracellular portion of the α-klotho protein is short (11 amino acids), while the extracellular portion is long (980 amino acids). The membrane fraction is also relatively short (21 amino acids)[96].

The extracellular portion contains two repeating sequences, termed KL1 (about 450 amino acids) and KL2 (about 430 amino acids). In the kidney and choroid plexus of the brain, the membrane protein can be hydrolyzed to produce 130 kDa, a soluble form of alpha-klotho protein, which is released into the circulation and cerebrospinal fluid, respectively. In humans, the secreted form of klotho is more diffuse than the membrane form[97].

Klotho is a transmembrane protein that, in addition to other effects, provides some control over an organism's sensitivity to insulin and appears to be involved in aging. Its discovery was documented in 1997 by Makoto Kuro-o Et al[98].The name of the gene comes from Klotho or Klotho, one of the Moirai, or Fates, in Greek mythology, who spins the thread of human life. The klotho protein is a β-glucuronidase capable of hydrolyzing steroid β-glucuronides. Genetic variants in KLOTHO have been associated with human aging, and klotho protein has been shown to be a wandering factor that can be detected in serum and decreases with age.The binding of endocrine fibroblast growth factors (FGF's, i.e., FGF19 and FGF21) to fibroblast growth factor receptors, is enhanced by their interactions as co-receptors with β-klotho. Loss of β-Klotho abolishes all effects of FGF21[99].

α-Klotho, which binds to the endocrine FGF FGF23 alters cellular calcium homeostasis, by increasing the expression and activity of TRPV5 (decreasing phosphate reabsorption in the kidney) and decreasing TRPC6 uptake (decreasing phosphate uptake from the intestine). α-Klotho increases calcium reabsorption in the kidney by stabilizing TPRV5. About 95% to 98% of the Ca2+ that is filtered from the blood by the kidneys is naturally reabsorbed by the renal tubules, which is mediated by TRPV5figure. 1-11 [100].

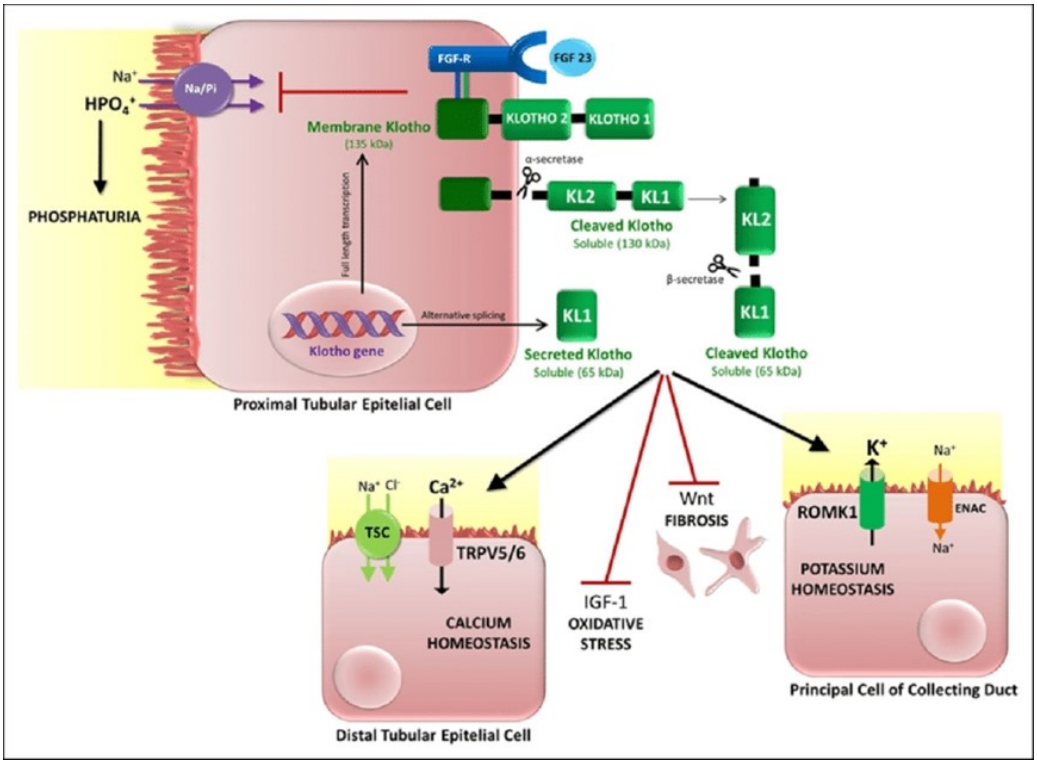


Figure 1-12 Main functions of the Klotho protein[101]..

1.5.2 Clinical Significance of the Klotho

α-klotho can suppress oxidative stress and inflammation, thereby reducing endothelial dysfunction and atherosclerosis. Plasma alpha klotho is increased by aerobic exercise, thus reducing endothelial dysfunction.β-klotho activation of FGF21 has a protective effect on cardiomyocytes. Obesity is characterized by resistance to FGF21, believed to result from inhibition of β-klotho by tumor necrosis

factor-alpha (a cytokine), but there is evidence against this mechanism.Klotho is essential for the maturation of oligodendrocytes, the integrity of myelin, and can protect neurons from toxic effects [102].

Decreased klotho expression is shown in pulmonary macrophages of smokers. An abnormal form of autophagy associated with reduced expression of klotto is associated with chronic disease. (Although normal autophagy helps maintain muscle, excessive autophagy leads to loss of muscle mass). It has been found that reduced klotho expression may be due to DNA hypermethylation, which may be caused by overexpression of DNMT3a. Klotho may be a reliable gene for early detection of methylation changes in oral tissues, and could be used as a target for therapeutic modification in oral cancer during early stages [103].

Klotho-deficient is accelerated human aging and exhibit extensive and accelerated atherosclerosis. In addition, they showed impaired endothelium-dependent vasodilation and impaired angiogenesis, suggesting that klotho protein may protect the cardiovascular system through the production of endothelium-derived nitric oxide. Klotho can play a protective role in Alzheimer's disease patients [104].

Decreased α-klotho or FGF23 can lead to impaired excretion of phosphate by the kidneys, resulting in hyperphosphatemia.The plasma (soluble) form of α-klotho is readily measured and has been shown to decrease after 40 years of age in humans. Lower plasma levels of α-klotho in the elderly is associated with increased frailty and all-cause mortality [105].

It is proven that physical activity increases plasma alpha klotho. In addition, variations in the klotho gene (SNP Rs9536314) are associated with longer life and increased cognition in humans, but only if gene expression is heterozygous, not homozygous. The cognitive benefits of α-klotho are primarily seen late in life. The presence of senescent cells reduces alpha klotho levels. Antigens reduce the level of these cells, allowing alpha klotho levels to increase [106].

Aim of the Study

1-To investigate the level of Klotho, PTH and bone-markers in patients with long-term use of glucocorticoid

2-To evaluate the correlation between serum level of Ca^{+2},PTH and Klotho in patients using glucocorticoid more than 3 months

3-Assessment of circulating klotho in study the effect of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis .

4-Evaluation of the correlation between serum level of calcium, PTH and klotho in study the effect of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis.

Chapter Two

Materials and Methods

2. Materials and Methods

2.1. Study Design

The study was designed as a case-control study.

2.2 Materials

2.2.1 Chemical and kits

The chemicals and kits in the present study were used as supplied from purchases without additional purification. Kits and chemicals used in the present study are shown in Table 2-1.

Table 2-1. The Chemicals and Kits

No.	CHEMICALS	COMPANY AND COUNTRY
1	PTH ELISA Kit	CUSABIO.(USA)
2	Calcium Kit	RANDOX (UK)
3	Klotho ELISA Kit	CUSABIO. (USA)

2.2.2 Instruments and Equipment

The instruments and equipment used in this study are listed in Table 2-2.

Table 2-2. Instruments and Equipment's Used

No.	Instruments and Equipment	Company and Country
1	0.1ml pipette tips	China
2	1ml pipette tips	China
3	Centrifuge EBA 20	Hettich/Germany
4	Deep Freezer	Samsung/Korea
5	Disposable cuvette China	China
6	Disposable syringe (5 ml)	China
7	ElIZA washer, reader and printer	Biotek /USA
8	Eppindorf tube (0.5 ml)	China
9	Kan tube	China
10	Micropipettes (5-50µl),(100-1000µl)	Slamed / Germany
11	Multichannel micropipette	Slamed / Germany
12	Refrigerator	agur/Turkish
13	Spectrophotometer	Jenway/Italian
14	Tank for multichannel micropipette	China
15	Test tube with Separating gel	AFCO , Jordan
16	Water Bath	HH-2 Chain

2.3. Subjects

2.3.1. The Place and Date of Study

This study was carried out in the department of Chemistry and Biochemistry, college of medicine at university of Babylon. The samples were collected from the registered attendances of the Rheumatology Clinic/Murjan Teaching Hospital, Al-Imam Al-Sadiq Hospital, and Al-Hilla Teaching Hospital during the period from 1st of December 2022 until 1st of March 2023. The patients were diagnosed by specialist physician.

2.3.2 Patients Group

The sample size of the patient group in the present study was selected according to Daniel's sample size formula equation[107]. Consist of 50 patients (19 male and 31female).

2.3.3 The Diagnosis of Patients Group

In this study, the diagnosis of the effect of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients was confirmed by a detailed questionnaire was filled out, that contains (age, sex, weight, height, long term use of glucocorticoid for more than 3 months and with osteoporosis (by diagnosis DXA examination of -2.5 degree)).

2.3.4. Control Group

A sample of control group of 50 look healthy individuals (19 men and 31females) was collected . whom matched with patients in terms of sex and age. They were obtained from health volunteer without any history of osteoporosis.

2.3.5 . Inclusion Criteria

Persons were proven with long term use of glucocorticoid for more than 3 months and with osteoporosis (by diagnosis DXA examination of -2.5 degree) and sample taken within the age from (20 – 50) years mean age (39.31 ± 2.07) were in the current study as a patient group, in addition to apparently healthy individuals as control group within the age from (21-51) years mean age (39.37 ± 1.81).

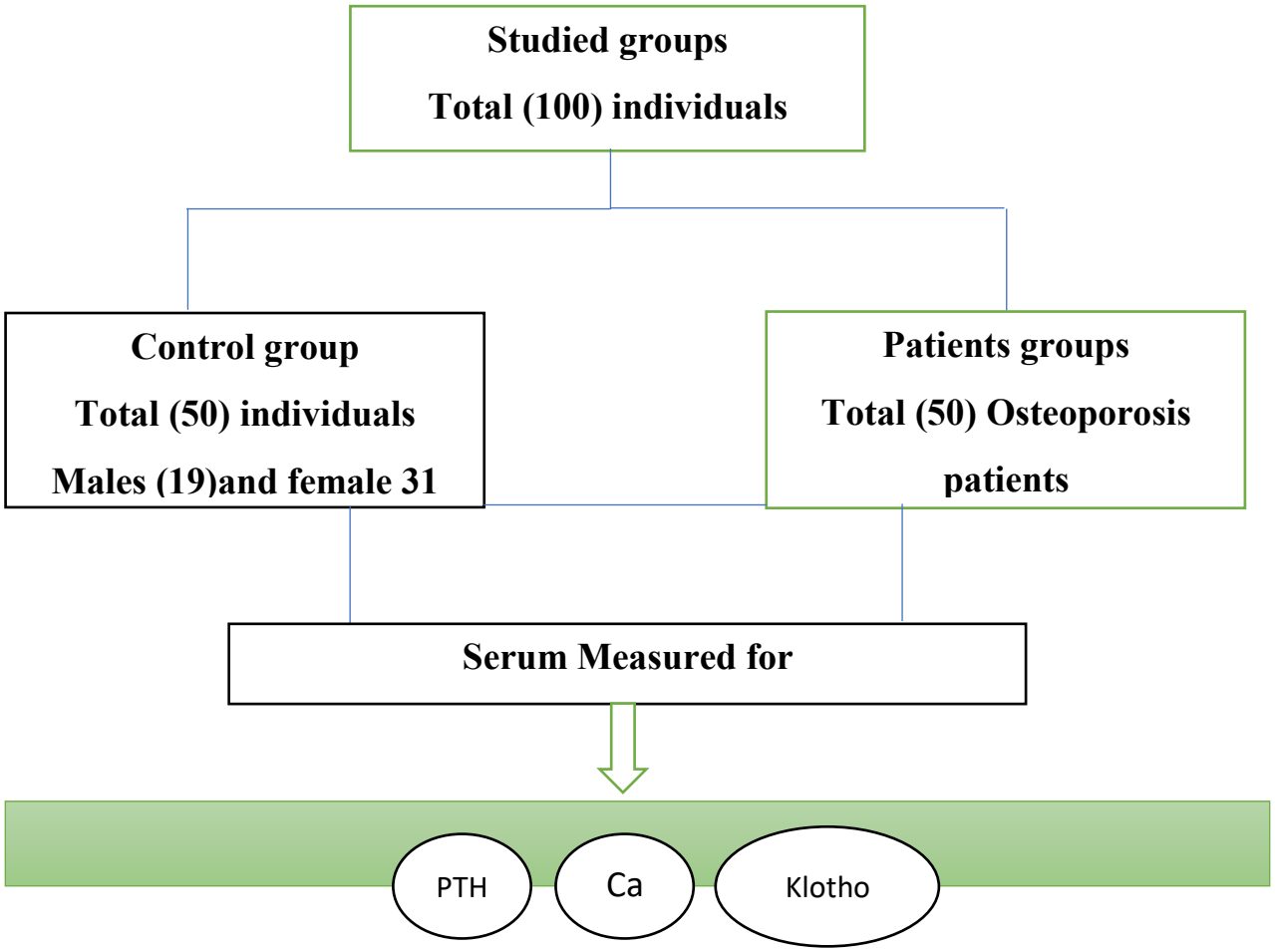


Figure 2-1. Studied groups

2.3.6. Exclusion Criteria

The persons have inherited metabolic abnormality, pregnant, and breastfeeding women.

2.3.7. Ethical Issues

Ethical issues depends on the following:

1-Approval by a scientific committee in the College of medicine (University of Babylon, Iraq) and the Biochemistry Department in the same college.

2-The aims and procedure of this study were clarified to all participant in the present study to gain their verbal acceptance.

3- Ethical and scientific committee in hospital.

2.4. Methods

2.4.1. Collection of Blood Sample

A blood sample was collected from the vein of each participant using a needle puncture approximately (5ml) was placed in disposable tubes containing separating gel. The blood sample in the gel tube was kept at room temperature for five minutes to coagulate. And later it centrifuged for (10 minutes) at Xg subsequently, then pipetting the transparent serum into three clear dry Eppendorf tubes and stored at (-20℃) until used for the several examinations. The serum was thawed at (20-25℃) temperature for 1 hours then submitted to the centrifuge for 5 minute at Xg .

2.4.2. Body Mass Index (BMI)

Body mass index was measured in all individuals based on a weight-to square height ratio derived by achieving a mathematical equation that divided the weight in kilograms by the square height in meters , and the results were considered as follows:

1-Underweight ≤ (18.5).

2-Normal weight (18.5 - 24.9).

3. Overweight (25-29.9).

4. Obese ≥ (30).

BMI (Kg/m^2) = weight (Kg) / height (m^2) [108]

2.5. Human Parathyroid Hormone PTH ELISA Kit

2.5.1 Measurement Human Parathyroid Hormone PTH ELISA Kit

2.5.2 Principle

The quantitative sandwich enzyme immunoassay method is used in this assay. A microplate has been pre-coated with a PTH specific antibody. Pipette standards and samples into the wells, and any PTH present is bound by the immobilized antibody. After eliminating any unattached compounds, a biotin-conjugated antibody specific for PTH is applied to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is applied to the wells. A substrate solution is added to the wells after a wash to remove any unbound avidin-enzyme reagent, and color develops in proportion to the quantity of PTH bound in the initial step. The color development is terminated, and the color intensity is measured.

Detection range 62.5 pg/ml-4000 pg/ml.

Sensitivity: less than 15.6 pg/ml.

Specificity : No significant cross-reactivity or interference between human PTH and analogues was observed.

2.5.3. Material

2.5.3.1 Reagents Quantity

Assay plate 1(96 coated Microwells)

Standard (Freeze dried) 2vial

Biotin antibody (100 x concentrate) 1vial x 120 µl

HRP avidin (100 x concentrate) 1vial x 120 µl

Biotin antibody Diluent 1vial x 15 ml

HRP avidin Diluent 1vial x 15 ml

Sample Diluent 1vial x 50 ml

Wash Buffer (25 x concentrate) 1vial x 20 ml

TMB Substrate 1vial x 10 ml

Stop Solution 1vial x 10 ml

Adhesive Strip (For 96 wells) 4

2.5.3.2 Reagent Preparation

All of the reagents have been used. Before usage, allow 30 minutes at room temperature (18-25^{o}C).

1. Biotin-antibody had been centrifuged before the vial is opened. Then a 100x. dilution was prepared. As the following: 990 µl of Biotin-antibody Diluent. +10 µl of Biotin-antibody.

2. HRP-avidin before the vial was opened. It had been centrifuged then a 100-fold dilution was prepared. As the following: 990 µl of HRP-avidin Diluent+10 µl of HRP-avidin.

3. Wash Buffer: To avoid crystals forming in the concentrate, it is warmed to room temperature and mixed gently until the crystals have completely dissolved 20 ml of Wash Buffer Concentrate (25 x) was diluted into deionized or distilled water to prepare 500 ml of Wash Buffer (1 x).

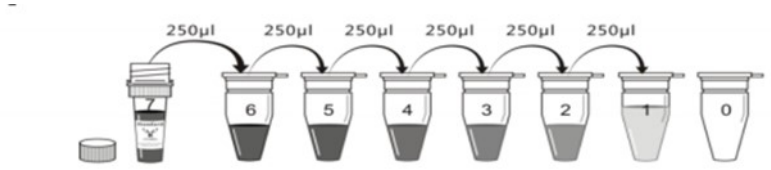
2.5.3.3 Standard

Standard vial centrifuged for 30 seconds at Xg . solution of 4000 pg/ml was prepared by reconstituting the standard with 1.0 ml of sample diluent.

A volume of 250 µl of sample diluent was pipetted into each tube (S0-S6).

The stock solution is used to produce a 2-fold dilution series (below) by Mix each tube thoroughly before the next transfer.

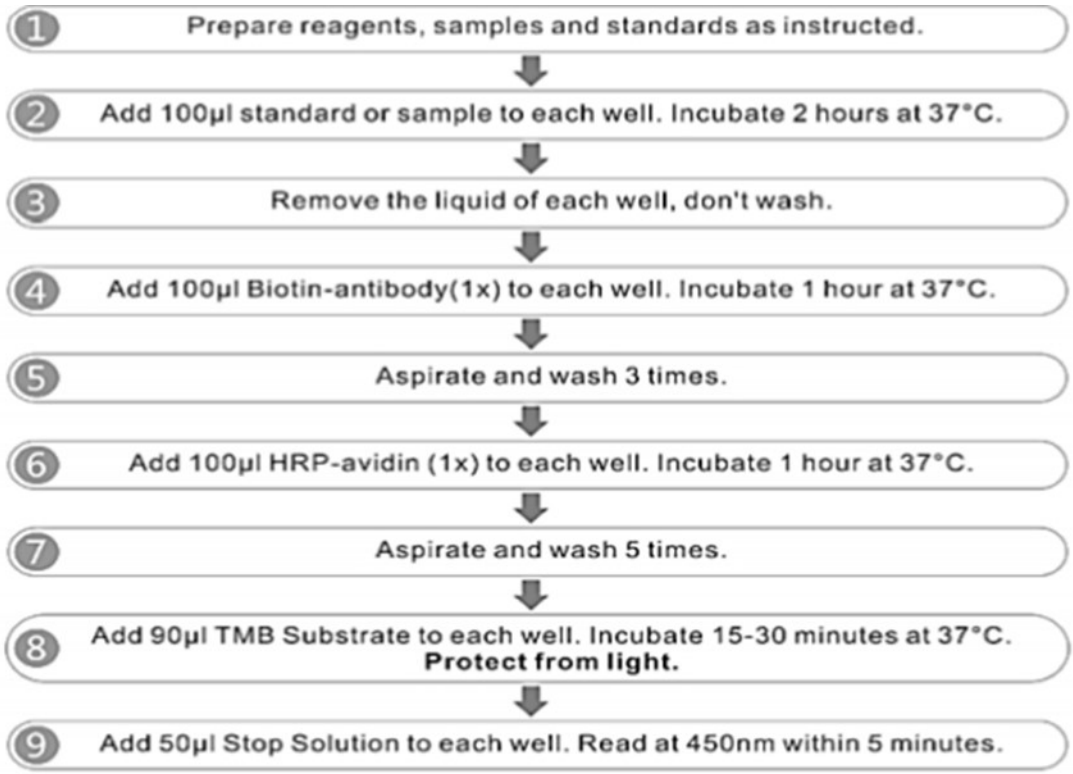
The undiluted Standard serves as the high standard (4000 pg/ml). Sample Diluent serves as the zero standard (0) pg/ml.



Tube	S7	S6	S5	S4	S3	S2	S1	S0
pg/ml	4000	2000	1000	500	250	125	62.5	0

Figure 2-2. Serial dilution of standard solution of PTH

2.5.4. Steps of PTH measurement



2.5.5. Calculation of results

Results were calculated by Using the professional soft "Curve Expert" to make a standard curve.

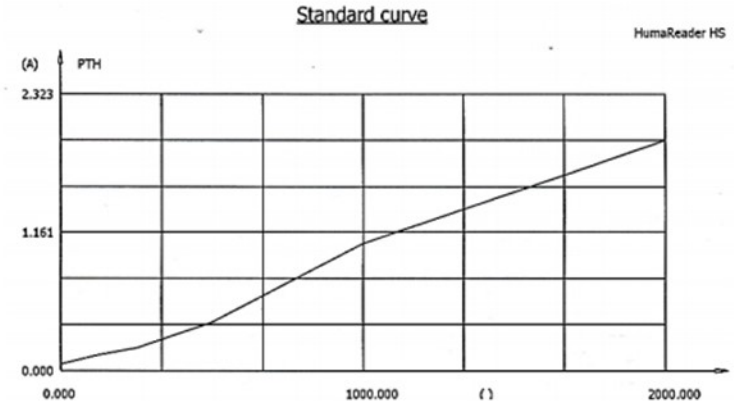


Figure 2-3. Standard curve PTH test

2.6. Calcium

2.6.1 Determination of Serum Calcium

2.6.2 Principle

Calcium ions form a violet complex with O-Cresolphthalein complexone in analkaline medium, that absorbs at 570nm

Calcium + O-Cresolphthalein Complexone $\xrightarrow[\text{Medium}]{\text{Alkaline}}$ Calcium-Cresolphthalein Complexone

Complex (purple color).

2.6.3 Assay procedure

	Blank	Standard	Sample
Standard		20ml	
Sample			20ml
Working Reagent (R1+R2)	1ml	1ml	1ml

Equal volumes of color and buffer reagent was combined, mixed and stand for twenty minutes at room temperature then used and incubated for 1minute at room temperature. The absorbance (A) of standard and sample was read against the blank at 570 nm.

2.6.4 Calculations

Calcium (mg/ dl) = $\frac{A\ Sample}{A\ Standard}$ x Standard conc (10)

*According to this procedure, the reference values for serum calcium concentration were 8.5-10.5mg/dl.

2.7 Human Klotho ELISA Kit

2.7.1Measurment Human Klotho ELISA Kit

2.7.2 Principle

The quantitative sandwich enzyme immunoassay method is used in this assay. A microplate has been pre-coated with a Klotho specific antibody. Pipette standards and samples into the wells, and any Klotho present is bound by the immobilized antibody. After eliminating any unattached compounds, a biotin-conjugated antibody specific for Klotho is applied to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is applied to the wells. A substrate solution is added to the wells after a wash to remove any unbound avidin-enzyme reagent, and color develops in proportion to the quantity of Klotho bound in the initial step. The color development is terminated, and the color intensity is measured.

Detection range: 0.156 ng/ml-10 ng/ml.

Sensitivity: less than 0.039 ng/ml.

Specificity : No significant cross-reactivity or interference between human Klotho

and analogues was observed.

2.7.3 Material

2.7.3.1Reagents Quantity

Assay plate 1(96 coated Microwells)

Standard (Freeze dried) 2vial

Biotin antibody (100 x concentrate) 1vial x 120 µl

HRP avidin (100 x concentrate) 1vial x 120 µl

Biotin antibody Diluent 1vial x 15 ml

HRP avidin Diluent 1vial x 15 ml

Sample Diluent 1vial x 50 ml

Wash Buffer (25 x concentrate) 1vial x 20 ml

TMB Substrate 1vial x 10 ml

Stop Solution 1vial x 10 ml

Adhesive Strip (For 96 wells) 4

2.7.3.2 Reagent prepration

All of the reagents have been used. Before usage, allow 30 minutes at room temperature (18-25^{o}C)

1- Biotin-antibody had been centrifuged before the vial is opened. Then a 100x dilution was prepared. As the following: 990 µl of Biotin-antibody Diluent. +10 µl of Biotin-antibody.

2- HRP-avidin before the vial was opened it had been centrifuged then A 100-fold dilution was prepared. As the following: 990 µl of HRP-avidin Diluent+10 µl of HRP-avidin.

3- Wash Buffer: To avoid crystals forming in the concentrate, it is warmed to room temperature and mixed gently until the crystals have completely dissolved 20ml of Wash Buffer Concentrate (25 x) was diluted into deionized or distilledwater to prepare 500 ml of Wash Buffer (1 x)

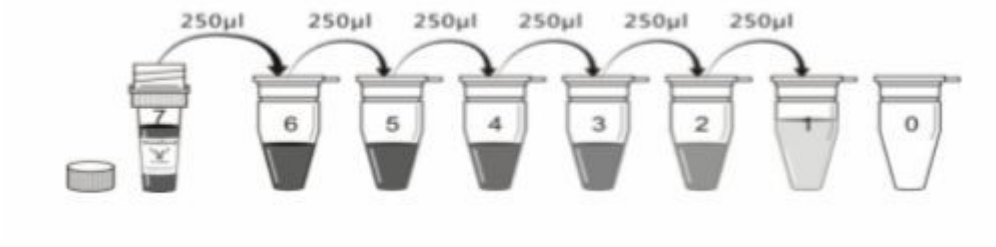
2.7.3.3 Standard

Standard vial centrifuged for 30 seconds at Xg solution of 10 ng/ml was prepared by reconstituting the standard with 1.0 ml of sample diluent.

A volume of 250µl of sample diluent was pipetted into each tube (S0-S6).

The stock solution is used to produce a 2-fold dilution series (below) by Mix each tube thoroughly before the next transfer.

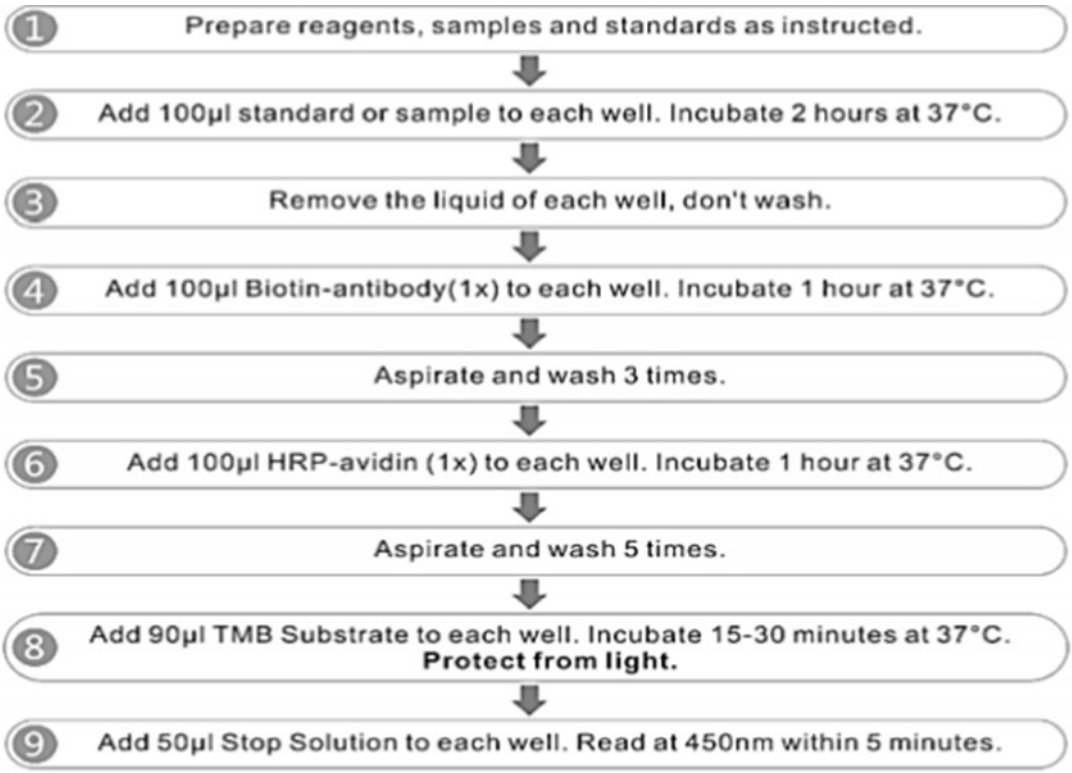
The undiluted Standard serves as the high standard (10 ng/ml). Sample Diluent serves as the zero standard (0 ng/ml .



Tube	S7	S6	S5	S4	S3	S2	S1	S0
ng/ml	10	5	2.5	1.25	0.625	0.312	0.156	0

Figure 2-4. Serial dilution of standard solution of Klotho

2.7.4 Steps of Klotho measurement



2.7.5 Calculation of results

Results were calculated by Using the professional soft "Curve Expert" to make a standard curve.

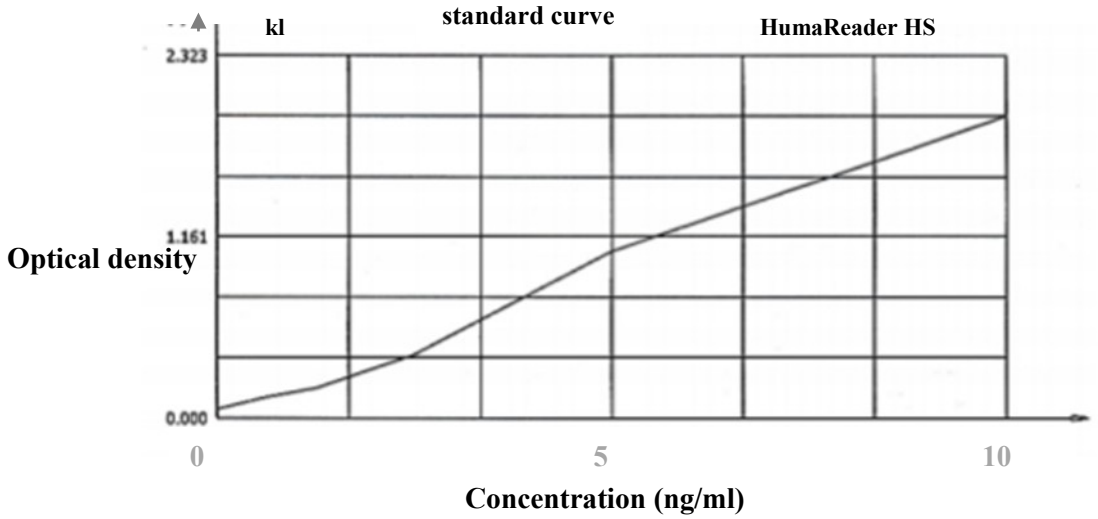


Figure 2-5. Standard curve Klotho

2.8 Statistical Analysis

Statistical analyses were performed using SPSS statistical package for Social Sciences (version 26.0 for Windows, SPSS, Chicago, IL, USA). Data normality distribution was examined using the Shapiro-Wilk test. Calcium, Klotho, and PTH distribution were considered normal. Student's t test and ANOVA tests were used to test differences in studied parameters within different groups. Quantitative data are represented as mean, standard deviation. Qualitative data are represented as count and percentage. Scatterplots was used for visual demonstrating of relationships between independent variables. Pearson correlation conducted for measurement of correlation coefficients and their significance. ROC survival test was used for evaluating the ability of study markers to discriminate disease from nondisease subjects. A p-value of <0.05 was considered statistically significant[109].

Chapter three

Results and Discussion

3. Results and Discussion

3.1 Demographic and Clinical Characteristics of the Study Group

3.1.1 Distribution of Study Groups According to Sex

The sex distribution of the studied groups was 50 patients with long term use of glucocorticoid suffer from osteoporosis, 19 (38%) male and 31(62%) female, matching with controls and the results represented in figure. 3-1.

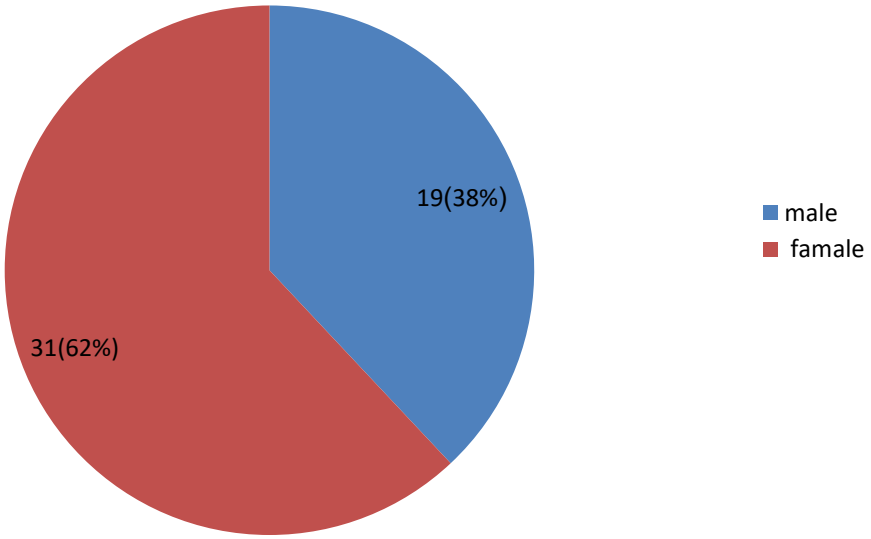


Figure 3-1. Sex distribution of patients and controls

3.1.2 Age

In this study, 100 participants were divided into 50 patients and other healthy people, and their ages ranged between 20 and 50 years. A comparison between study groups regarding age was performed using a t-test there was not statistically significant difference P 0.980. The dispersion of age the rate malady is appeared in Table 3-1 and figure 3-2, the mean age of patients of (39.31 ± 2.07) years compared with healthy control (39.37 ± 1.81) years P 0.980.

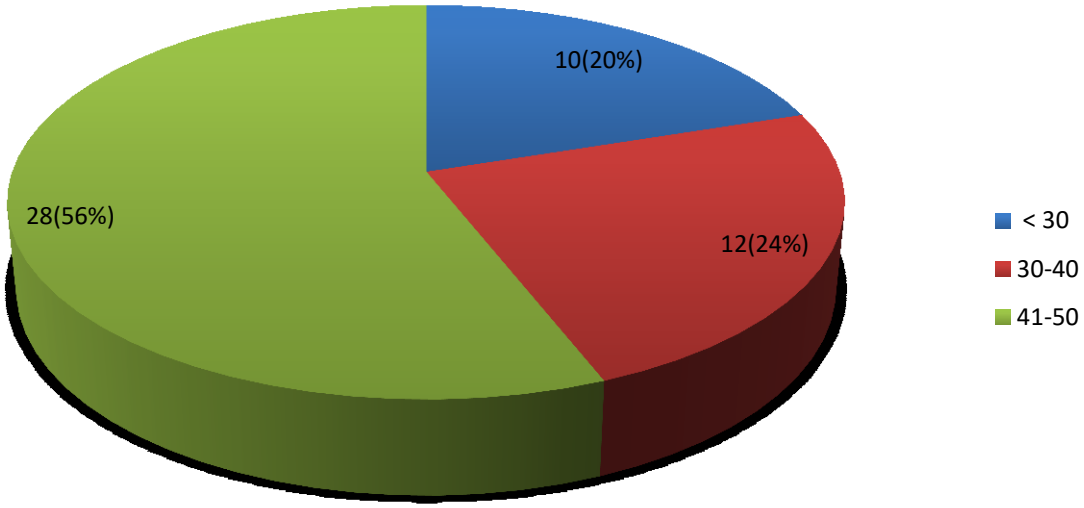


Figure 3-2. Age distribution of patients

According to the results of this study which found more cases of long term use of glucocorticoid patients in (41-50 years)age that agree with previous study of Ranasinghe, Kumara *etal*. The most susceptible age group was between the ages of 40 and 50 [110].

3.1.3 Comparison Body Mass Index (BMI) between Patients and Healthy Groups

Results of present study showed that the BMI was significantly higher in patients ($p \leq 0.05$) when the compared to control. as illustrated in Table. 3-2.

The use of glucocorticoids drugs cause increase in weight and this agree with the Patients use glucocorticoids drugs current study and different from the patients don't use glucocorticoids drugs current study.

Table 3-1 . Comparison between patients and control in BMI

Variable	Patients, Mean ± SD	Control ,Mean ± SD	P-value
BMI (kg/m2)	28.99 ± 0.58	26.96 ± 0.8	0.0494*
* (P≤0.05)			

3.2 Examination the Effect of Biochemical Parameters in the Study Groups

3.2.1 Study The Effect of Parathyroid Hormone PTH

Estimation of PTH in patients and control groups were representing in figure. 3-3 (They show that the mean and SD for long term use of glucocorticoid patients and control were (858.03 ± 42.62 pg/ml),(613.84 ± 23.12 ng/ml) respectively so that PTH was significantly (P≤0.01) higher in long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients compared with control.

Table 3-2. Comparison between Patients and Control in PTH Level

Variable	Patients, Mean ± SD	Control ,Mean ± SD	P-value
PTH (ng /ml)	858.03 ± 42.62	613.84 ± 23.12	0.0001**
** ($P \leq 0.01$)			

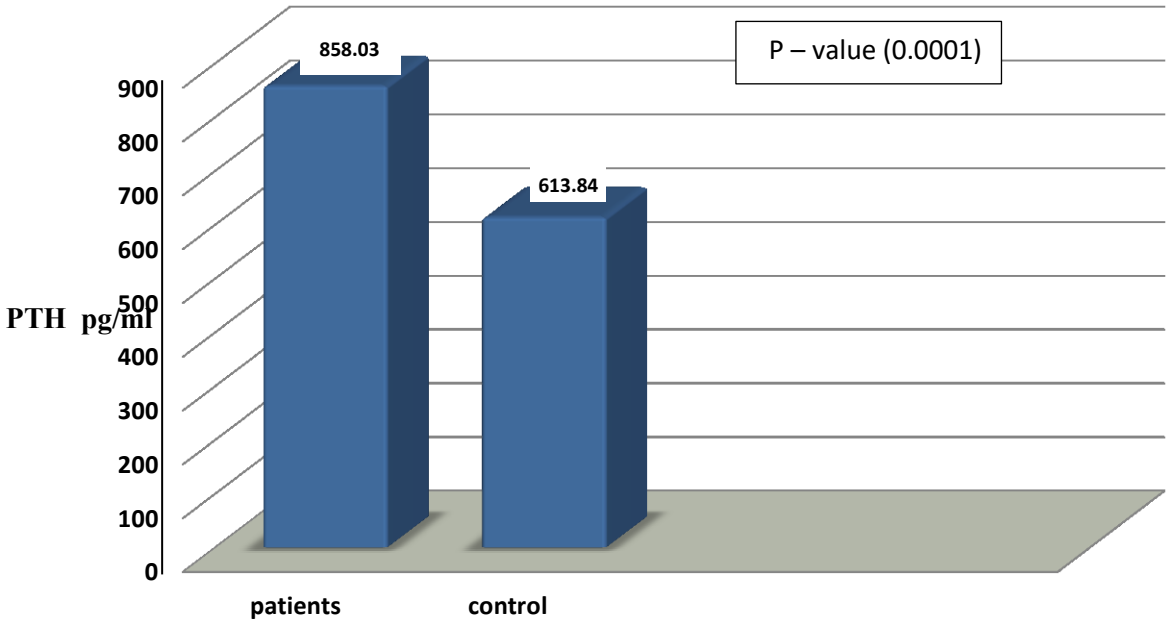


Figure 3-3. Comparison between patient and control in PTH

Long term use of glucocorticoids causes hypocalcemia and osteoporosis by impairing vitamin D activation, leading in a compensatory increase in parathyroid gland cellularity and parathyroid hormone synthesis and secondary hyperparathyroidism (SHP) [111]. Mnazzal and Abdullah indicate that PTH levels were significantly higher in the patient group (858.03 ± 42.62) pg/ml compared with healthy control group (613.84 ± 23.12) pg/mL[112]. Isakova, Cai *etal*. alshow that the long term use of glucocorticoid patients, PTH level is elevated somewhat, but calcium levels decreased slightly[113], that agrees with the current study results.

3.2.2 Study the Effect of Calcium on The Study Groups

The current study shows highly significant decrease in calcium levels between patients and healthy control (P≤0.01), as shown in Table. 3-3.

Table 3-3. Comparison between Patients and Control in Calcium Level

Variable	Patients, Mean ± SD	Control ,Mean ± SD	P-value
Ca (mg /dl)	7.34 ± 0.16	8.98 ± 0.08	0.0001**
** (P ≤ 0.01)			

This study shows that the mean and SD of the calcium level for patients was (7.34 ± 0.16) and for healthy control was (8.98 ± 0.08), respectively. Calcium was significant lower in long term use of glucocorticoid control. The most common complication occurring in chronic use of glucocorticoids is osteoporosis. Ca homeostasis is hormonally controlled by a four-tissue axis comprising the gut, bone, kidney, and parathyroid gland, that closely regulate serum Ca levels within a restricted range. The primary two hormones involved in calcium balance are parathyroid hormone (PTH) and klotho [114]. Hypocalcemia stimulates PTH secretion and hypercalcemia suppresses it. Calcium-sensing receptors (CaSR) on the parathyroid gland detect low levels of calcium in the blood, which stimulates PTH synthesis and secretion. Hypocalcemia, secondary hyperparathyroidism (SHPT) occur [115]. The biochemical changes of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis include raised parathyroid hormone (PTH) and decreased serum calcium[116]. The results of the current study are agreed Keung and Perwad [117], DiMeglio and Imel [118], Barreto *etal*. [119], that found significantly lowe plasma Ca levels and in patients with long term use of glucocorticoid compared with healthy controls.

3.2.3 Study the Effect of Klotho

The klotho estimation in patients and control groups were representing in figure3-4. They show that the mean and SD levels for patients and control were (607.03 ±31.62 , 333.84 ± 16.11) ,(respectively so that klotho was significantly(P≤0.01) higher in long term use of glucocorticoid patients compared to control.

Table 3-4. Comparison between Patients and Control in klotho Level

Variable	Patients, Mean ± SD	Control ,Mean ± SD	P-value
klotho (ng /ml)	607.03 ±31.62	333.84 ±16.11	0.0001**
** (P ≤ 0.01)			

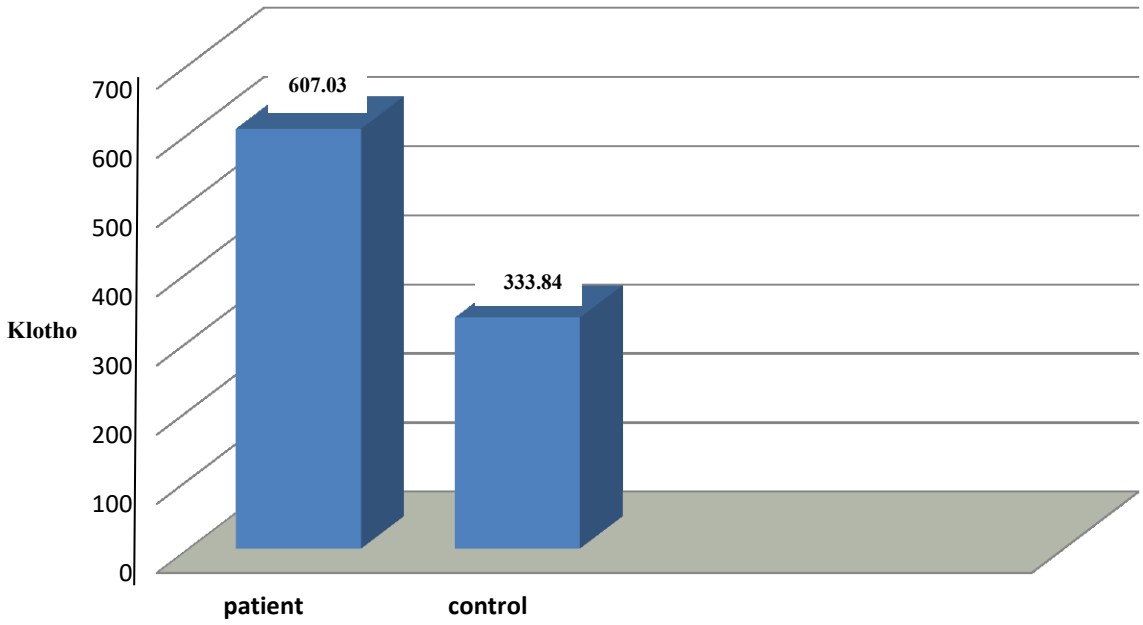


Figure 3-4. Klotho levels in the samples of patient and controls

Klotho and parathyroid hormone are hormones that regulate renal calcium reabsorption and vitamin D metabolism As renal function diminishes in long-term use of glucocorticoid, circulating klotho and PTH concentrations rise Secondary hyperparathyroidism is a component of long-term use of glucocorticoid inducing osteoporosis [120].

3.3 Study the Correlation between the Biomarkers

3.3.1 Study the Correlation between PTH and other Parameter

The result of the current study showed significant correlation between PTH with Ca ($p<0.01$) for diagnosis long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis and PTH with new biomarker Klotho ($p<0.01$) showed in a table. 3-4 and figure. 3-5.

Table 3-5 Correlation between PTH and other parameter

	Parameter	Person correlation (r)	P-value
PTH	Ca	0.32	0.0017
	Klotho	0.38	0.0002

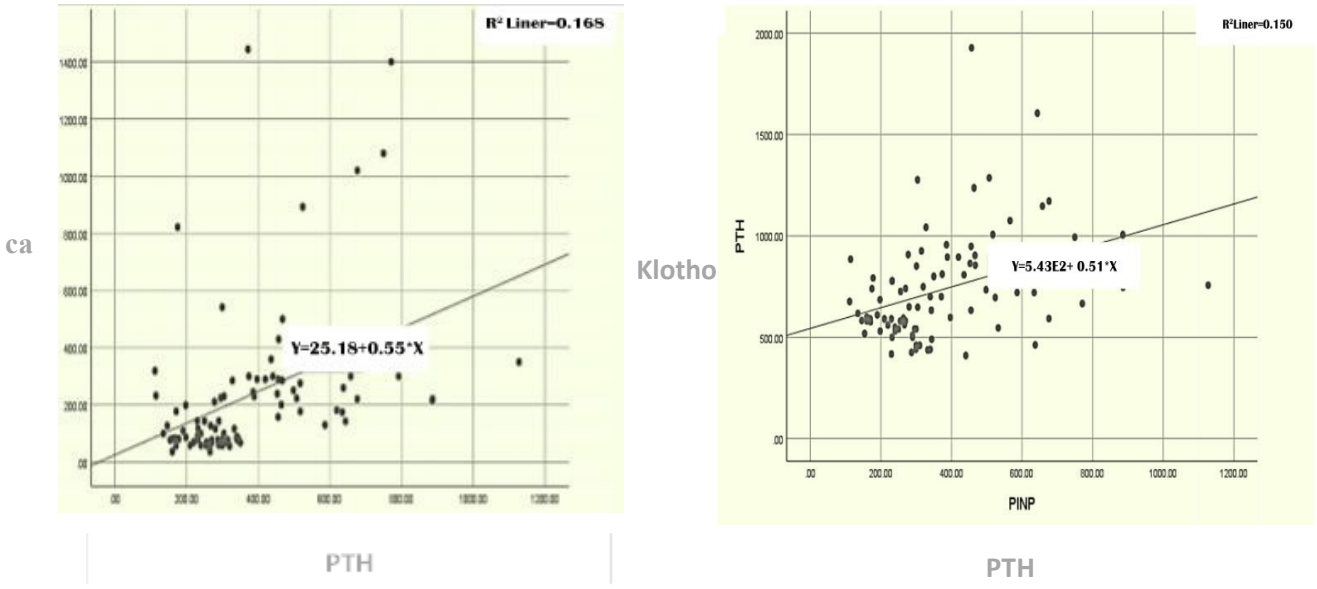


Figure 3-5. The correlation between PTH and other parameters (Ca, klotho) among long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients.

3.3.2 Study the Correlation Between Klotho and other parameter

The result of the current study showed significant correlation between Klotho with ca ($p<0.01$) for diagnosis long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis and Klotho with biomarker PTH($p<0.01$) showed in a table. 3-5 and figure. 3-6.

Table 3-6 Correlation between Klotho and other parameter

	Parameter	Person correlation (r)	P-value
Klotho	PTH	0.49	0.0001
	Ca	0.56	0.0001

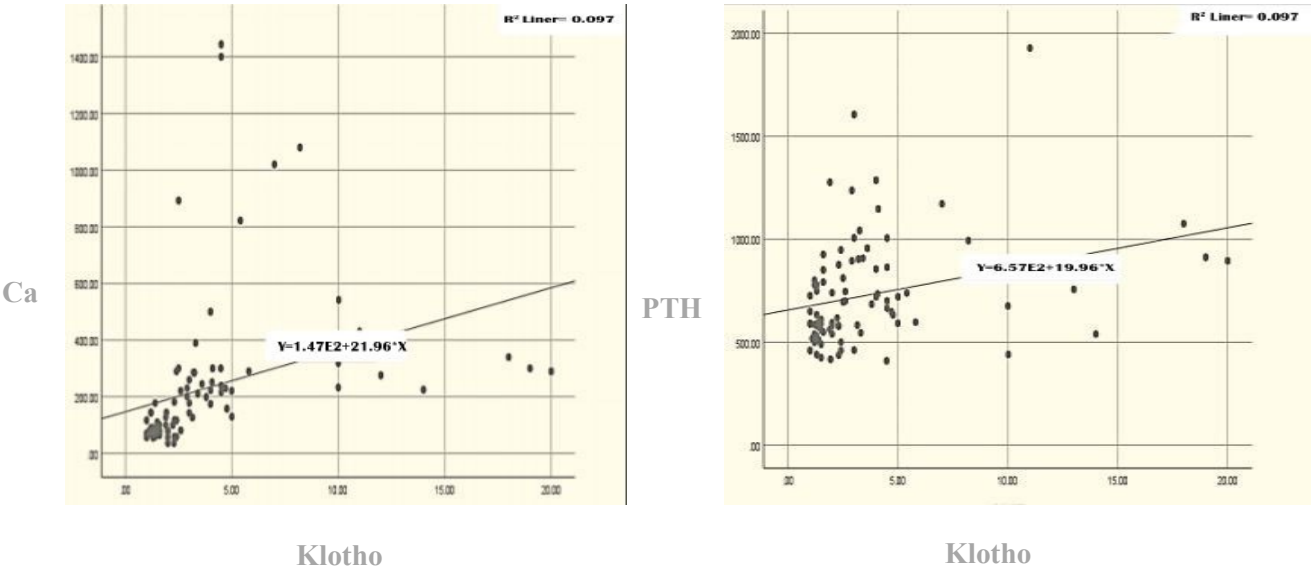


Figure 3-6. The correlation between Klotho and other parameters (Ca, PTH) among long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients.

3.3.3 Study the Correlation Between Calcium and other parameter

The result of the current study showed significant correlation between Ca with PTH ($p<0.01$) for diagnosis long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis and calcium with new biomarker klotho ($p<0.01$). showed in a Table. 3-6

Table 3-7 Correlation between Ca and other parameters

	Parameter	Person correlation (r)	P-value
Ca	PTH	0.49	0.0001
	Klotho	0.38	0.0002

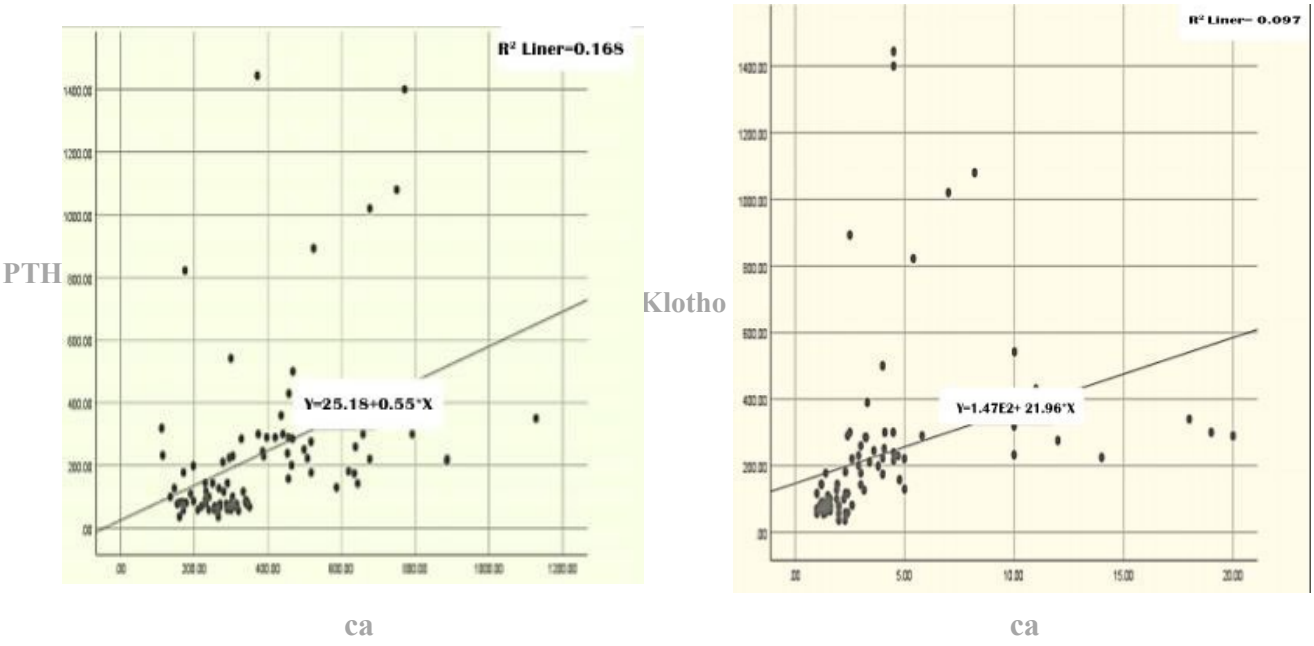


Figure 3-7. The correlation between Calcium and other parameters (PTH, klotho) among long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients.

3.4 ROC Curve of Biochemical Parameters

The receiver operating characteristic curve (ROC curve) was used to evaluate the diagnostic values of biomarkers in discrimination between long term use of glucocorticoid patients and controls, as shown in table 3-7

Table 3-8 ROC analysis of PTH and Klotho markers as bone turnover marker of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis from Control.

marker	SE	Cut-off	SN%	SP%	AUC	p-value
PTH	0.03	≥ 198.1	77.8%	84.4%	0.82	0.0001
Klotho	0.18	≥ 26.9	75.7%	88.0%	0.962	0.003
SN: sensitivity, SP: specificity, AUC: area under curve, P-val.: p value						

3.4.1 ROC Analysis of Klotho

ROC curve for the sensitivity and specificity of Klotho (ng\ml) for diagnosis of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis compare with control , (Cut-off point was >26.9 ng/ml), AUC=0.962 , P= 0.003, the sensitivity and the specificity were 75.7 %, 88.0 % respectively, indicating a fair discriminative value, as shown in figure 3-6.

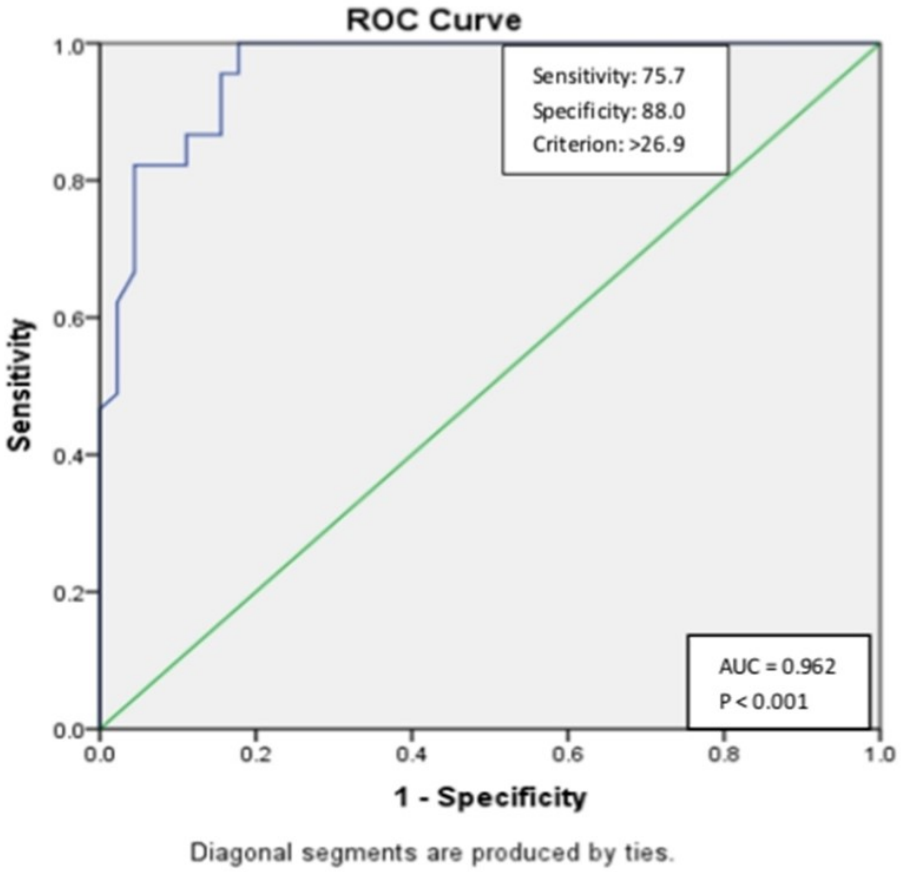


Figure 3-8. ROC Curve For Klotho

3.4.2 ROC Analysis of PTH

Figure 3-9. shows the ROC curve between long term use of glucocorticoid patients and controls. The test revealed that the area under the curve (AUC) was 0.82, p=0.001. The sensitivity and specificity of the test at the cut-off value of ≥ 198.1 pg/ml were 77.8% and 84.4% ,respectively.

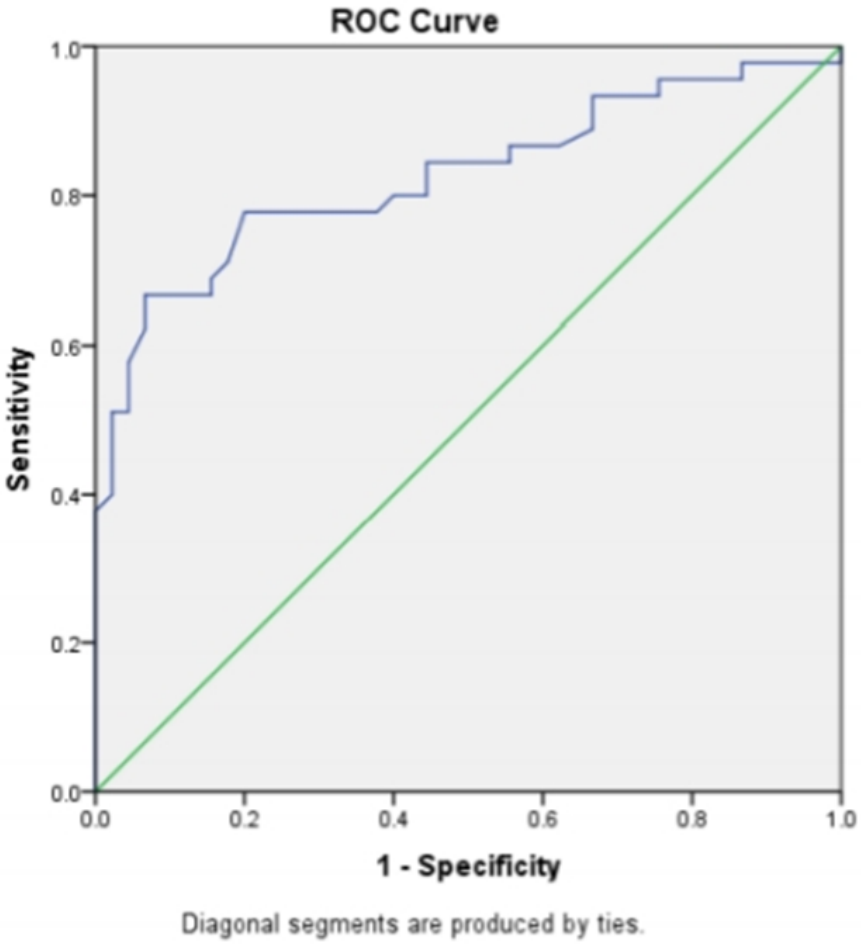


Figure 3-9. ROC Curve For PTH

Conclusions and Recommendations

Conclusions:

This study concludes that :

1- The current study revealed, there is low Ca^{+2} level and high level of PTH and Klotho in patients on long term use of glucocorticoid

2- Ca^{+2},PTH and Klotho can help in detection of Osteoporosis in patients with long-term usage of glucocorticoid and can be useful in follow up of this conditiong osteoporosis.

Recommendations :

1- Future studies performed with a larger size of sample .
2- The follow-up study on the same patients yields a more accurate picture of the variability in the levels of PTH, Ca, and Klotho.
3- Estimate Klotho, PTH, and Ca as bone turnover parameter in long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis patients before and after treatment for bone minral disease.
4- Uses Klotho, PTH, and Ca with other biomarkers related to bone trunover CTX, and osteocalcin in futuer studies to be more knowledgeable about bone mineral status and to have a better understanding of the pathophisylogy of long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis
5- Assess bone turnover biomarkers in correlation with long term use of glucocorticoid on bone and its mechanism of inducing osteoporosis stagings.

References

References

References:

[1] Six M, Morin C, Fardet L. Association between prescription of long-term systemic glucocorticoid therapy associated measures and prescriber's medical speciality. 2019;40:427–32.

[2] McNamara KM, Kannai A, Sasano H. Possible roles for glucocorticoid signalling in cancer. Mol Cell Endocrinol. 2018;466:38–50.

[3] Reducing corticosteroid use in rheumatoid arthritis (Press Release), Berlin, Germany, July 31, 2020, EurekAlert! Accessed August 17, 2020.

[4] Mohamad, N.V.; Ima-Nirwana, S.; Chin, K.Y. Therapeutic potential of annatto tocotrienol with self-emulsifying drug delivery system in a rat model of postmenopausal bone loss. Biomed. Pharmacother. 2021, 137, 111368.

[5] Mazzantini M, Di Munno O. Glucocorticoid-induced osteoporosis: 2019 update. Reumatismo. 2020;66:144–52.

[6] Adcock IM, Mumby S. Glucocorticoids. Handb Exp Pharmacol. 2019;237:171–96.

[7] Russell G, Lightman S. The human stress response. Nat Rev Endocrinol. 2019 Sep;15(9):525-534.

[8] Kannai A, Lightman S., Human stress response. Nat Rev Endocrinol. 2019 Sep;16(7):526-533.

[9] Exton JH. Regulation of gluconeogenesis by glucocorticoids. Monogr Endocrinol. 2019;12:535-46.

[10] Fichna M, Fichna P. Glucocorticoids and beta-cell function. Endokrynol . 2018;68(5):568–73.

References

[11] Nieman LK. Cushing's syndrome: update on signs, symptoms and biochemical screening. Eur JEndocrinol. 2019Oct;173(4):M33-8.

[12] Cain DW, Cidlowski JA. Immune regulation by glucocorticoids. Nat Rev Immunol (2018) 17:233–47.

[13] Coutinho AE, Chapman KE. The anti-inflammatory and immunosuppressive effects of glucocorticoids, recent developments and mechanistic insights. Mol Cell Endocrinol. 2020Mar 15;335(1):2-13.

[14] Messina OD, Vidal LF, Vidal MV, Bultink IEM, Raterman HG, Lems W. Management of Glucocorticoid-Induced Osteoporosis. Aging Clin Exp Res (2021) 33:793–804.

[15] Chung LP, Upham JW, Bardin PG, Hew M. Rational oral corticosteroid use in adu72lt severe asthma: a narrative review. Respirology. 2020; 25(2): 161.

[16] Liu, S.; Zhou, L.; Yang, L.; Mu, S.; Fang, T.; Fu, Q. Gastrodin alleviates glucocorticoid induced osteoporosis in rats via activating the Nrf2 signaling pathways. Oncotarget 2018, 9, 11528–11540.

[17] Tang, Y.H.; Yue, Z.S.; Li, G.S.; Zeng, L.R.; Xin, D.W.; Hu, Z.Q.; Xu, C. Effect of β-ecdysterone on glucocorticoid-induced apoptosis and autophagy in osteoblasts. 2018, 17, 158–164.

[18] Shi, C.; Huang, P.; Kang, H.; Hu, B.; Qi, J.; Jiang, M.; Zhou, H.; Guo, L.; Deng, L. Glucocorticoid inhibits cell proliferation in differentiating osteoblasts by microRNA-199a targeting of WNT signaling. J. Mol. Endocrinol. 2018, 54, 325–337.

References

[19] Cawthorn, W.P.; Scheller, E.L.; Parlee, S.D.; Pham, H.A.; Learman, B.S.; Redshaw, C.M.; Sulston, R.J.; Burr, A.A.; Das, A.K.; Simon, B.R.; et al. Expansion of Bone Marrow Adipose Tissue During Caloric Restriction Is Associated With Increased Circulating Glucocorticoids and Not WithHypoleptinemia. Endocrinology 2019, 157, 508–521.

[20] Sato, A.Y.; Richardson, D.; Cregor, M.; Davis, H.M.; Au, E.D.; McAndrews, K.; Zimmers, T.A.; Organ, J.M.; Peacock, M.; Plotkin, L.I.; et al. Glucocorticoids Induce Bone and Muscle Atrophy by Tissue-Specific Mechanisms Upstream of E3 Ubiquitin Ligases. Endocrinology 2018, 158, 664–677.

[21] Bellido T, Plotkin LI, Bruzzaniti A. Chapter 3 - Bone Cells. In: Burr DB, Allen MR, editors. Basic and Applied Bone Biology, 2nd ed. Academic Press (2019). p. 37–55.

[22]Straub, R.H.; Cutolo, M.; Pacifici, R. Evolutionary medicine and bone loss in chronic inflammatory diseases--A theory of inflammation-related osteopenia. Semin. Arthritis Rheum. 2018, 45, 220–228 .

[23] Gilbert, M.P.; Pratley, R.E. The impact of diabetes and diabetes medications on bone health. Endocr. Rev. 2018, 36, 194–213 .

[24] Liu, S.; Fang, T.; Yang, L.; Chen, Z.; Mu, S.; Fu, Q. Gastrodin protects MC3T3-E1 osteoblasts from dexamethasone-induced cellular dysfunction and promotes bone formation via induction of the NRF2 signaling pathway. 2018, 41, 2059–2069.

[25] Elvy Suhana, M.; Fairus, A.; Norazlina, M.; Mohamad Fairuz, Y.; Ima Nirwana, S. Protective Effects of Palm Tocotrienol Against Glucocorticoid Induced Osteoporosis via Regulation of Gene Expressions. 2018, 13, 175–197.

References

[26] Suhana, M.R.; Fairus, A.; Nur Izrin, S.; Ahmad, A.I.; Chen Hee, C.; Sanghari, N.K.; Izam, A.M.; Fairuz, Y.; Nirwana, S.I. Beneficial Effects of Annatto (Bixa Orellana) Tocotrienol on Bone Histomorphometry and Expres-sion of Genes Related to Bone Formation and Resorption in Osteoporosis Induced by Dexamethasone. 2018, 103, 85–100.

[27] Shanbhogue, V.V.; Mitchell, D.M.; Rosen, C.J.; Bouxsein, M.L. Type 2 diabetes and the skeleton: New insights into sweet bones. Lancet Diabetes Endocrinol. 2019, 4, 159–173.

[28] Zhang, S.; Li, D.; Yang, J.Y.; Yan, T.B. Plumbagin protects against glucocorticoid-induced osteoporosis through Nrf-2 pathway. Cell Stress Chaperones 2018, 20, 621–629.

[29]Kobza, A.O.; Herman, D.; Papaioannou, A.; Lau, A.N.; Adachi, J.D. Understanding and Managing Corticosteroid-Induced Osteoporosis. Open Access Rheumatol. 2021, 13, 177–190.

[30] Zhao, B.; Luo, M.; Wang, A.; Zhang, W. MiR-182 antagonist alleviates glucocorticoid-induced secondary bone degeneration and osteoclast differentiation. 2022, 67, 123–130.

[31] Conaway, H.H.; Henning, P.; Lie, A.; Tuckermann, J.; Lerner, U.H. Activation of dimeric glucocorticoid receptors in osteoclast progenitors potentiates RANKL induced mature osteoclast bone resorbing activity. Bone 2019, 93, 43–54.

[32] Fu, L.; Wu, W.; Sun, X.; Zhang, P. Glucocorticoids Enhanced Osteoclast Autophagy Through the PI3K/Akt/mTOR Signaling Pathway. Calcif. Tissue Int. 2020, 107, 60–71.

References

[33] Chen, Y.H.; Peng, S.Y.; Cheng, M.T.; Hsu, Y.P.; Huang, Z.X.; Cheng, W.T.-K.; Wu, S.-C. Different susceptibilities of osteoclasts and osteoblasts to glucocorticoid-induced oxidative stress and mitochondrial alterations. Chin. J. Physiol. 2019, 62, 70–71.

[34] Varacallo M, Seaman TJ, Jandu JS, Pizzutillo P. StatPearls Publishing; Treasure Island (FL): Osteoporosis Sep 4, 2022.

[35] Kanis JA, Johansson H, Harvey NC, McCloskey EV. A brief history of FRAX. Arch Osteoporos. 2018 Oct 31;13(1):118.

[36] Walzak LC, Loken Thornton W. The role of illness burden in theory of mind performance among older adults. Exp Aging Res. 2018 Oct-Dec;44(5):427-442.

[37] Tsukasaki, M. & Takayanagi, H. Osteoimmunology: evolving concepts in bone-immune interactions in health and disease.19, 626–642 2019 .

[38] Jandu Js , Harvey Nc, Peng Sy . Stat pearls Publishing ; treasure Island (FL) : Juvenilc osteoporosis June 3,2022 .

[39] Wenjing, Jiayao, et al. “JAK2/STAT3 regulates estrogen-related senescence of bone marrow stem cells.” Journal of Endocrinology vol 245 (2020): 141-153.

[40] J Christopher Gallagher, “Vitamin D and aging” Endocrinol Metab Clin North Am. 2019 Jun;42:319-32.

[41] Rupee. “Medications that affect calcium.” Endocrine Practice Mar-Apr 2018;17 Suppl 1:26-30.

[42] Nieves, “Osteoporosis: the role of micronutrients” The American Journal of Clinical Nutrition, Volume 81, Issue 5, May 2019, Pages 1232–1239.

References

[43] Egeberg, A.; Schwarz, P.; Harslof, T.; Andersen, Y.M.F.; Pottegard, A.; Hallas, J.; Thyssen, J.P. Association of Potent and Very Potent Topical Corticosteroids and the Risk of Osteoporosis and Major Osteoporotic Fractures. JAMA Derm. 2021, 157, 275–282.

[44] Andersen, Y.M.F.; Pottegard, A.; Hallas, J.; Thyssen, J.P. Association of Potent and Very Potent Topical Corticosteroids and Osteoporosis Fractures. JAMA Derm. 2021, 157, 275–282.

[45] Kang, S.; Han, M.; Park, C.I.; Jung, I.; Kim, E.H.; Boo, Y.J.; Kang, J.I.; Kim, S.J. Use of serotonin reuptake inhibitors and risk of subsequent bone loss in a nationwide population-based cohort study. Sci. Rep. 2021, 11, 13461.

[46] Kokorovic, A.; So, A.I.; Serag, H.; French, C.; Hamilton, R.J.; Izard, J.P.; Nayak, J.G.; Pouliot, F.; Saad, F.; Shayegan, B.; et al. Canadian Urological Association guideline on androgen deprivation therapy: Adverse events and management strategies. Can Urol. Assoc .J. 2021, 15.

[47] Napoli, N.; Conte, C.; Pedone, C.; Strotmeyer, E.S.; Barbour, K.E.; Black, D.M.; Samelson, E.J.; Schwartz, A.V. Effect of Insulin Resistance on BMD and Fracture Risk in Older Adults. J. Clin. Endocrinol. Metab. 2019, 104, 3303–3310.

[48] Mbewe, C., Chanda-Kapata, P., Sunkutu-Sichizya, V., Lambwe, N., Yakovlyeva, N., Chirwa, M., Ayele, B & ,.Pitcher, R. D. " An audit of licenced Zambian diagnostic imaging equipment and personnel". Pan African Medical Journal. 1-14-2021.

[49] Strotmeyer, E.S.; Barbour, K.E.; Black, D.M.; Samelson, E.J.; Schwartz, A.V. Effect of Insulin Resistance on BMD and Fracture Risk in Older Adults. J. Clin. Endocrinol. Metab. 2019, 104, 3304–3311.

[50] Kokorovic, A.; So, A.I.; Serag, H.; French, C.; Hamilton, R.J.; Izard, J.P.; Nayak, J.G.; Pouliot, F.; Saad, F.; Shayegan, B.; et al. Canadian Urological

References

Association guideline on androgen deprivation therapy: Adverse events and management strategies. Can Urol. Assoc. J. 2021, 15, E307–E322.

[51] Koshizaka, M.; Ishikawa, K.; Ishibashi, R.; Maezawa, Y.; Sakamoto, K.; Uchida, D.; Nakamura, S.; Yamaga, M.; Yokoh, H.; Kobayashi, A.; et al. Effects of ipragliflozin versus metformin in combination with sitagliptin on bone and muscle in Japanese patients with type 2 diabetes mellitus: Subanalysis of a prospective, randomized, controlled study (PRIME-V study). J. Diabetes Investig. 2021, 12, 200–206.

[52] Yang, B.R.; Cha, S.H.; Lee, K.E.; Kim, J.W.; Lee, J.; Shin, K.H. Effect of dipeptidyl peptidase IV inhibitors, thiazolidinedione, and sulfonylurea on osteoporosis in patients with type 2 diabetes: Population-based cohort study. Osteoporos. 2021, 32, 1705–1712.

[53] U.S. Food & Drug Administration. FDA Drug Safety Communication: Possible Increased Risk of Fractures of the Hip, Wrist, and Spine with the Use of Proton Pump Inhibitors. drugs/postmarket-drug-safety-information-patients-and-providers/fda-drug-safety-communication-possible-increased-risk-fractures-hip-wrist-and-spine-use-proton pump#TableofEpidemiologicalStudiesevaluatingfractureriskwithprotonpumpinhibitors (accessed on 15 November 2021).

[54] Signorelli, S.S.; Scuto, S.; Marino, E.; Giusti, M.; Xourafa, A.; Gaudio, A. Anticoagulants and Osteoporosis. Int. J. Mol. Sci. 2019, 20, 5275.

[55] Monov, S.V.; Monova, D.; Shumnalieva, R. THU0500|Effects of long-term use of unfractionated heparin (UFH) or low-molecular-weight heparin (LMWH) on bone mineral density (BMD) in patients with nephrotic syndrome. 2018, 77, 456.

References

[56] Yang, S.; Niu, Q.; Gan, L.; Zhang, X.; Tu, L.; Zuo, L. Effect of long-term use of unfractionated or low-molecular-weight heparin on bone mineral density in maintenance hemodialysis patients. Hemodial. 2020, 24, 374,382.

[57] Yokoyama, S.; Ieda, S.; Nagano, M.; Nakagawa, C.; Iwase, M.; Hosomi, K.; Takada, M. Association between oral anticoagulants and osteoporosis: Real-world data mining using a multi-methodological approach. 2020, 17, 471–479.

[58] Marques, J.V.O.; Nalevaiko, J.Z.; Oliveira, M.F.; Raetsch, A.W.P.; Marques, G.L.; Petterle, R.R.; Moreira, C.A.; Borba, V.Z.C. Trabecular bone score (TBS) and bone mineral density in patients with long-term therapy with warfarin. Arch. Osteoporos. 2020, 15, 102.

[59] Nalevaiko, J.Z.; Marques, J.V.O.; Oliveira, M.F.; Raetsch, A.W.P.; Marques, G.L.; Petterle, R.R.; Moreira, C.A.; Borba, V.Z.C. Bone density and quality in patients treated with direct-acting oral anticoagulants versus warfarin. Bone 2021, 150, 116000.

[60] Hans SK, Levine SN. StatPearls Publishing; Treasure Island (FL): May 22, 2022. Hypoparathyroidism.

[61] Lofrese JJ, Basit H, Lappin SL.StatPearls Publishing; Treasure Island (FL): Jul 18, 2022. Physiology, Parathyroid.

[62]Goyal A, Anastasopoulou C, Ngu M, Singh S. StatPearls Publishing; Treasure Island (FL): Jul 24, 2022. Hypocalcemia.

[63] Rudin AV, McKenzie TJ, Wermer RA, Thompson GB, Lyden ML. Primary Hyperparathyroidism: Redefining Cure. Am Surg. 2019 Feb 01;85(2):214-218.

[64] Sharma S, Hashmi MF, Castro D.StatPearls Publishing; Treasure Island (FL): Sep 12, 2022. Hypophosphatemia.

References

[65] Byrd C, Kashyap S, Kwartowitz G. StatPearls Publishing; Treasure Island (FL): Jul 25, 2022. Parathyroid Cancer.

[66] Lofrese JJ, Basit H, Lappin SL. StatPearls Publishing; Treasure Island (FL): Jul 18, 2022. Physiology, Parathyroid.

[67] Gerlinger C, Oster M, Borgelt L, Reyer H, Muráni E, Ponsuksili S, Polley C, Vollmar B, Reichel M, Wolf P, Wimmers K. Physiological and Transcriptional Responses in Weaned Piglets Fed Diets with Varying Phosphorus and Calcium Levels. Nutrients. 2019 Feb 20;11(2).

[68] Silva BC, Cusano NE, Bilezikian JP. Primary hyperparathyroidism. Best Pract Res Clin Endocrinol Metab. 2018 Sep 28;:101247.

[69] Prades JM, Asanau A, Timoshenko AP, Gavid M, Martin C. Endoscopic parathyroidectomy in primary hyperparathyroidism. Eur Arch Otorhinolaryngol. 2018 Jun;268(6):893-7.

[70] Timoshenko AP, Gavid M, Martin C. Endoscopic parathyroidectomy in hyperparathyroidism. Eur Arch Otorhinolaryngol. 2018 Jun;268(9):891-7.

[71] Endo I, Matsumoto T, Fukumoto S. [Pathophysiology and classitication of parathyroidism]. Clin Calcium. 2019 Aug;17(8):1175-81.

[72] Sugarman JA, Douglass AM, Say EA, Shields CL. Stones, bones, groans, thrones, and psychiatric overtones: Systemic associations of sclerochoroidal calcification. Oman J Ophthalmol. 2018Jan-Apr;32(5):70-73.

[73] Laepin SL , Gavid M , Ngu M, bones, thrones, and psychiatric overtones: Systemic associations of sclerochoroidal calcification. Ophthalmol. 2018 Jan-Apr;10(1):47-49.

[74] Pitt SC, Sippel RS, Chen H. Secondary and tertiary hyperparathyroidism, state of the art surgical management. Surg Clin North Am. 2019 Oct;89(5):1227-39.

References

[75] Husebye ES, Perheentupa J, Rautemaa R, Kämpe O. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. J Intern Med. 2019 May;265(5):514-29.

[76] Kraus C, Vanicek T, Weidenauer A, Khanaqa T, Stamenkovic M, Lanzenberger R, Willeit M, Kasper S. DiGeorge syndrome : Relevance of psychiatric symptoms in undiagnosed adult patients. Wien Klin Wochenschr. 2018 Apr;130(7-8):283-287.

[77] Vakiti A, Mewawalla P. StatPearls. StatPearls Publishing; Treasure Island (FL): Jul 25, 2021. Hypercalcemia.

[78] Afzal M, Kathuria P. StatPearls. StatPearls Publishing; Treasure Island (FL): Jul 19, 2022. Familial Hypocalciuric Hypercalcemia.

[79] Sadiq NM, Naganathan S, Badireddy M. StatPearls Publishing; Treasure Island (FL): Sep 5, 2022. Hypercalcemia.

[80] Khan M, Jose A, Sharma S. Physiology, Calcium . [Updated 2022 Oct 29]. . Treasure Island (FL): StatPearls Publishing; 2022 Jan.

[81] " Calcium". Linus Pauling Institute, Oregon State University, Corvallis, Oregon. 31 August 2019.

[82] Sosa Torres, Martha; Kroneck, Peter M.H; "Introduction: From Rocks to Living Cells" pp. 1–32 in "Metals, Microbes and Minerals: The Biogeochemical Side of Life" (2021) pp. xiv + 341.

[83] Wermer RA, Thompson GB, Lyden ML. StatPearls Publishing; Treasure Island (FL): Jul 25, 2021. Hypercalcemia.

[84] Hluchan and Pomerantz, StatPearls Publishing; Treasure Island (FL): Jul 19, 2022. Familial Hypocalciuric Hypercalcemia.

References

[85] Naganathan S, Badireddy M. StatPearls Publishing; Treasure Island (FL): Sep 5, 2022. Hypercalcemia.

[86] Khan M, Jose A, Sharma S. Physiology, Parathyroid Hormone. [Updated 2022 Oct 29]. . Treasure Island (FL): StatPearls Publishing; 2022 Jan.

[87] "Calcium". Linus Pauling Institute, Oregon State University, Corvallis, Oregon. 31 August 2019.

[88] Ponsuksili S, Polley C, Vollmar B, Reichel M, Wolf P, Wimmers K. Physiological Responses in Weaned Piglets Fed Diets with Varying Calcium Levels. Nutrients. 2019 Feb 20;11(2).

[89] Martha; Kroneck, Peter M.H; "Introduction: From Rocks to Living Cells" pp. 1–32 in "Metals, Microbes and Minerals: The Biogeochemical Side of Life" (2021) pp. xiv + 341.

[90] McKenzie TJ, Thompson GB, Lyden ML. Hypercalcemia: Redefining Cure. Am Surg. 2019 Feb 01;85(2):214-218.

[91] Florenzano P, Hartley IR, Jimenez M, Roszko K, Gafni RI, Collins MT (2021) Tumor-induced osteomalacia. Calcif Tissue Int 108(1):128–142.

[92] Dolegowska K, Marchelek-Mysliwiec M, Nowosiad-Magda M, Slawinski M, Dolegowska B. "FGF19 subfamily members: FGF19 and FGF21". Journal of Physiology and Biochemistry. (June 2019) 75 (2): 229–240.

[93] Hanson K, Fisher K, Hooper NM (2021). "Exploiting the neuroprotective effects of α-klotho to tackle ageing- and neurodegeneration-related cognitive dysfunction". Neuronal Signaling. 5 (2).Levels. Nutrients. 2019 Feb 20;11(2).

[94] Dolegowska, K.; Marchelek-Mysliwiec, M.; Nowosiad-Magda, M.; Slawinski, M.; Dolegowska, B. FGF19 subfamily members :FGF19 and FGF21. J. Physiol. Biochem. 2019, 75, 229–240, doi:10.1007/s13105-019-00675-7 . Kurosu, H.; Kuro.

References

[95] Martín-González C, González-Reimers E, Quintero-Platt G, Martínez-Riera A, Santolaria-Fernández F. "Soluble α-Klotho in Liver Cirrhosis and Alcoholism". Alcohol and Alcoholism. (2019) 54 (3): 204–208.

[96] Kuro-O M . "The Klotho proteins in health and disease". Nature Reviews Nephrology. (2019) 15 (1): 27–44.

[97] Baranowska B, Kochanowski J. "The metabolic, neuroprotective cardioprotective and antitumor effects of the Klotho protein". Neuroendocrinology Letters. (2020) 41 (2): 69–75.

[98] Lim K, Halim A, Lu TS, Ashworth A, Chong I. "Klotho: A Major Shareholder in Vascular Aging Enterprises". International Journal of Molecular Sciences. (September 2019) 20 (18): E4637.

[99] Flippo KH, Potthoff MJ. "Metabolic Messengers: FGF21". Nature Metabolism. (2021) 3 (3): 309–317.

[100]van Goor MK, Hoenderop JG, van der Wijst J . "TRP channels in calcium homeostasis: from hormonal control to structure-function relationship of TRPV5 and TRPV6". Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. (2017) 1864 (6): 883–893.

[101] Keles N, Caliskan M, Dogan B, Keles NN, Kalcik M, Aksu F Kostek O, Aung SM, Isbilen B, Oguz A. Low serum level of Klotho is an early predictor of atherosclerosis. Tohoku J Exp Med. 2018;237(1):17–23 .Hu MC, Shi M.

[102] Torbus-Paluszczak M, Bartman W, Adamczyk-Sowa M . "Klotho protein in neurodegenerative disorders". Neurological Sciences. (2018) 39 (10): 1677–1682.

[103] Zhou H, Pu S, Zhou H, Guo Y. "Klotho as Potential Autophagy Regulator and Therapeutic Target". Frontiers in Pharmacology. (2021) 12: 755366.

References

[104] Lehrer S, Rheinstein PH. "Alignment of Alzheimer's disease amyloid β-peptide and klotho". World Academy of Sciences Journal. (2020) 2 (6).

[105] Veronesi F, Borsari V, Cherubini A, Fini M. "Association of Klotho with physical performance and frailty in middle-aged and older adults: A systematic review". Experimental Gerontology. (2021) 154: 111518.

[106] Lavars, Nick ."Senolytic drugs boost protein that protects against effects of aging". New Atlas. Retrieved 2022-03-16.

[107] ghrelin ASI, dan peningkatan berat badan bayi pada ibu menyusui obesitas dan normal Journal : Jurnal Gizi Klinik Indonesia, p. 22-27, july 2018.

[108] A. S. Singh and M. B. Masuku, “Sampling technique determination of sample size in applied statistics research: An overview,” Int. J. Econ. Commer. Manag, vol. 2, no. 11, pp. 1–22-2014 .

[109] Shafi MS, Faisal T, Naseem S, Javed S. Postgraduate Medical Train Understanding of Biostatistics: A Pre-and Post-Research Methodology Workshop Experience. US National Library of Medicine. 2018;28(3):196-200.

[110] Ranasinghe AV, Kumara G, Karunarathna RH, De Silva AP, Sachintani KGD, Gunawardena J, et al. The incidence, prevalence and treatmente of Chronic Disease and ChronicDisease of uncertain aetiology (CDu) in the North Central Province of Sri Lanka: an analysis of 30,566 patients. BMC.338:(1)20;2019

[111] Habas E, Sr., Eledrisi M, Khan F, Elzouki AY. Secondary Hyperparathyroidism in Chronic Disease: Pathophysiology and Management. Cureus. 2021;13(7):e16388.

[112] Mnazzal AH, Abdullah HN. Role of 1α-Hydroxylase Enzyme and 25 (OH (D as a Biomarker of Vitamin D Metabolism in Osteoporosis. Al-Nisour Journal for Medical Sciences. 2019;1(2).

References

[113] Isakova T, Cai X, Lee J, Mehta R, Zhang X, Yang W, et al. Longitudinal evolution of markers of mineral metabolism in patients with osteoporosis: The Bone disease American Journal of Bone and Kidney Diseases.

4-235:(2)75;2020

[114] Viegas C, Araújo N, Marreiros C, Simes D. The interplay between mineral metabolism, vascular calcification and inflammation in Chronic Disease

Osteoporosis: challenging old concepts with new facts. Aging . (AlbanyNY)

.4274:(12)11;2019

[115] Reiss AB, Miyawaki N, Moon J, Kasselman LJ, Voloshyna I, D'Avino Jr R ,et al. CD, arterial calcification, atherosclerosis and bone health: interrelationships and controversies. Atherosclerosis. 2018;278:49-59.

[116]Hill Gallant KM, Spiegel DM. Calcium balance in chronic disease .Current osteoporosis reports. 2017;15(3):214-21.

[117] .Keung L, Perwad F. Vitamin D and kidney disease. Bone reports

.100-9:93;2018

[118].DiMeglio LA, Imel EA. Calcium and phosphate: hormonal regulation and

metabolism. Basic and applied bone biology. 2019:257-82.

[119] Barreto FC, Barreto DV, Massy ZA, Drüeke TB. Strategies for phosphate control in patients with osteoporosis. Bone international reports. 2019;4(8):1043-56.

[120] Mousa D, Helal I, Alhejaili F, Alghamdi S, Alhweish A, Alhomrany M, et al. Sun-137 osteoporosis disease markers in Asia : population based pilot study. Bone International Reports. 2020;5(3):S258.

APPENDIX

Questionnaire

Case No. Date :

Hospital name :

Patient name-:

Age-:

Sex-:

Weight: - Kg

BMI-:

Height: - cm

Telephone number -:

Profession-:

Residence-:

Duration of disease-:

Final diagnosis of the disease-:

Other disease

الخلاصة:

الجلايكورتيكويدات هي واحدة من تلك الأدوية التي تستخدم في علاج الحالات المختلفة، مثل الحساسية والربو والتهاب المفاصل والنقائل السرطانية. مع الاستخدام طويل الأمد للجلوكوكورتيكويدات، خاصة الجرعات العالية طويلة المدى، قد تظهر سلسلة من التأثيرات الضارة. وتشمل هذه هشاشة العظام، وارتفاع السكر في الدم، ومقاومة الأنسولين، وارتفاع ضغط الدم، والعدوى الشديدة، ومتلازمة تشبه كوشينغ، والقرحة الهضمية، والاضطرابات العصبية والنفسية. الجلايكورتيكويدات هي السبب الأكثر شيوعا لهشاشة العظام الثانوية، ما يسمى بهشاشة العظام الناجمة عن الجلايكورتيكويد. الجهات الفاعلة الأساسية في تأثير الاستخدام طويل الأمد للجلوكوكورتيكويد لتحفيز هشاشة العظام تشمل هرمون الغدة الدرقية (PTH) والكالسيوم وكلوثو.

هدف الدراسة هو دراسة العلاقة بين مستويات PTH، كلوثو، والكالسيوم في الدم لدى المرضى الذين يعانون من استخدام طويل الأمد لهشاشة العظام القشرانية السكرية والضوابط الصحية. وقد تم تصميم هذه الدراسة كدراسة الحالات والشواهد. شارك في هذه الدراسة 50 مريضا (31 أنثى و 19 ذكرا) بالإضافة إلى 50 ضابطا (31 أنثى و 19 ذكرا) متطابقين تماما مع المرضى من حيث العمر والجنس.

مؤشر كتلة الجسم (BMI) كان (24-27)(كجم/م2 للمرضى وكان عمرهم (20-50) سنة متوسط العمر (39.31 ± 2.07) مؤشر كتلة الجسم مع (26.96 ± 0.8) كجم/م2 للمرضى السيطرة. مأخوذة من عيادات مستشفى مرجان التعليمي، مستشفى الامام الصادق، ومستشفى الحلة التعليمي.

تم قياس مستويات PTH وklotho بتقنية ELISA بينما تم قياس مستوى الكالسيوم بالطريقة اللونية حسب دليل الشركة المصنعة. كما أظهرت نتائج الاختبارات التي أجريت أن مستويات الكالسيوم مع الاستخدام طويل الأمد المسبب لهشاشة العظام كانت أقل بكثير من الأشخاص الأصحاء، حيث بلغت قيمة $P < 0.05$. كانت مستويات PTH وklotho في المرضى أعلى بكثير من الأشخاص الأصحاء، حيث كانت قيمة $P < 0.05$. أظهرت العلاقة وجود علاقة معنوية بين الكلوثو والكالسيوم في الاستخدام طويل الأمد للجلوكوكورتيكويد على العظام وآلية تحفيز مرضى هشاشة العظام. كان هناك ارتباط كبير بين كلوثو و PTH ($P \geq 0.01$). (كما كان هناك ارتباط كبير بين كلوثو والكالسيوم ($P < 0.001$) خلال الاستخدام طويل الأمد لمرضى الجلايكورتيكويد. أظهر اختبار منحنى خاصية تشغيل المتلقي قيمة تمييزية جيدة لكلوثو، و PTH بين الاستخدام طويل الأمد للجلوكوكورتيكويد في المرضى ومجموعة التحكم.ومع ذلك، أظهر اختبار منحنى خاصية تشغيل المستقبل قيمة تمييزية جيدة لكلوثو وPTH بين الاستخدام طويل المدى

للجلوكوكورتيكويد في المرضى ومجموعة التحكم.الدراسة الحالية التي أجريناها تقودنا إلى استنتاج مفاده أن كلوثو و PTH يمكن أن يكونا بمثابة علامة محتملة في الاستخدام طويل الأمد لمرضى الجلوكورتيكويد للتنبؤ بإمكانية الإصابة بهشاشة العظام عند الاستخدام طويل الأمد للجلوكوكورتيكويدات.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة بابل/ كلية الطب

قسم الكيمياء والكيمياء الحياتية والسريرية

دراسة تأثير الاستخدام طويل الأمد للجلوكوكورتيكويد على العظام وآليتها في إحداث هشاشة العظام في محافظة بابل

رسالة ماجستير

إلى مجلس كلية الطب في جامعة بابل للحصول على درجة الماجستير في العلوم الكيمياء الحياتية السريرية / الطب

من قبل

آيات جاسم كاظم اسعد

بكالوريوس/ المختبرات الطبية

2010 - 2011

اشراف

أ.د عبدالسميع حسن الطائي **أ.د عادل حسن علي**

2023 م **1444 هـ**