Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Misan
College of Science
Department of Biology

Evaluation of Osteocalcin Level, Some Hormones and Biochemical Criteria in Male Patients with Diabetes Mellitus Type 2 from Misan Province

A Thesis

Submitted to the Council of the College of Science/University of Misan as Partial Fulfillment of the Requirements for the Master Degree in Biology

By

Eman Ali Hussein Al-Saidy

B.Sc. Biology (2013)

Supervised by

Prof. Dr. Zainab Abdul Jabbar Ridha Al-Ali

September 2019 A.D **Muharram 1441 A.H**

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

﴿وَضَرَبَ لَنَا مَثَلًا وَنَسِيَ خَلْقَهُ ۖ قَالَ مَنْ يُحْيِي الْعِظَامَ وَهِيَ رَمِيم﴾

صدق الله العلي العظيم

(سورة يس: أيه ٧٨)

Supervisor's Certificate

We certify that this thesis entitled **"Evaluation of Osteocalcin Level, Some Hormones and Biochemical Criteria in Male Patients with Diabetes Mellitus Type 2 from Misan Province**

"has been prepared under my supervision at the College of Science, University of Misan; as a partial fulfillment of the requirements for the degree of Master of Biology

Signature

Prof. Dr. Zainab Abdul Jabbar Ridha Al- Ali

Department of Biology

College of Science/Misan University

Date: 16 / 9 / 2019

Recommendation of Head of Biology Department

I view of the available recommendations; I forward this thesis debate by the examining committee.

Signature

Assist. Prof. Dr.Zahid S. Aziz

Head of Department of Biology

College of Science/Misan University

Date: 16 / 9 / 2019

Dedication

To whom Allah sent as mercy to the Worlds

Prophet Mohammed

To the one who sacrificed his soul for Iraq

My marty brother Hassan

To who never stop giving of themselves in countless ways
My great parents

To Which stands by me when things look bleak

My beloved brothers and sister

With my love……Eman

Acknowledgment

All praises to Allah and his blessing for the completion of this thesis. I thank God for all the opportunities, trials and strength that have been showered on me to finish writing the thesis

First and foremost, I would like to sincerely thank my supervisor supervisor Prof. Dr. Zainab Abdul Jabbar Ridha for her guidance, understanding, and patience and most importantly, she has provided positive encouragement and a warm spirit to finish this thesis. It has been a great pleasure and honor for me to be my supervisor. I would particularly thanks to Dean of the College of Science and Head of departmentt of biology at the College of Science, University of Misan to give me the opportunity to join graduate studies.

I would like to thank Dr. Sana Kazem Khalaf from the University of Basrah; College of education pure sciences and Prof. Dr. Asaad Yehia from the University of Basrah, college of Agriculture for their assistance in statistical analysis. I would like to thank the staff of the Specialist Endocrinology Center for their helping me in obtaining patients and collecting samples.

Thanks also dedicated to all of the patients and healthy for their cooperation. I would like to take this opportunity to say thanks to all my friends, who have been so supportive along the way of doing my thesis. I also thank my family who encouraged me and prayed for me throughout the time of my research. Finally, I thank all who in one way or another contributed in the completion of this thesis.

Eman

Summary

Summary

The current study aimed to evaluation osteocalcin (OC) level, some hormones and biochemical criteria and fulfills from relationship among OC and parameters above in male patients with type 2 diabetes mellitus (T2DM) at Misan province during the period from December of 2018 to May of 2019. The study included 110 male (66 with T2DM and 44 healthy male) aged (35-49) years. The study sample was divided according to the age into three groups, the first (35-39) years, the second (40-44) years and the third (45-49) yearss. Also divided according to the the body mass index (BMI) to three groups, the normal weight (18.5-24.9 k/m^2), overweight (25-29.9 k/m^2) and obesity (BMI>30 k/m^2).

The results of the present study showed the values of fasting blood glucose (FBG), Glycosylated haemoglobin A1c (HbA1c), osteocalcin (OC) and insulin in patients with T2DM increased significantly ($P<0.05$) in comparision with control groups, while the values of prolactin in patients with T2DM decrease significantly ($P<0.05$) in comparision with control group.

The values of FBG and HbA1c increased significantly ($P<0.05$) in diabetics groups compared to the control groups for all age and BMI groups. The values of total calcium (Ca) in patients of second age and overweight groups increased significantly ($P<0.05$) compared to the control groups. The value of the OC hormone showed a significantly ($P<0.05$) increased in patients of the second age groups when compared to the control and to patients of first age group, also its values increased significantly ($P<0.05$) in

patients of overweight and obesity as compared to control groups. The value of insulin hormone increased significantly (P<0.05) in the first age group, normal weight and obesity compared to the control of same groups, also it's increased significantly (P<0.05) in patients of obesity compare to other patients groups.

The results showed that luteinizing hormone (LH) values in third age and overweight groups increased significantly (P<0.05) as compared to control of same groups, but the values of the LH and testosterone (TT) differed significantly (P<0.05) among patients with T2DM of age groups. While the value of prolactin (PRL) decreased significantly (P<0.05) in the first age and normal weight groups in patients with T2DM compared to control groups.The values of thyroid hormones and thyroid stimulating hormone did not differ significantly (P<0.05) between patients and control groups for all age and BMI groups. The value of parathyroid hormone (PTH) decreased significantly (P<0.05) in patients of the first age and normal weight groups compared to the control of same groups.The results showed no relationship between OC and other parameters studies

The conclusions of present study observed that the OC, insulin and PTH levels increase with age and BMI, while PRL concentration decreased in male with T2DM.

Table of Contents

List of tables

List of Figures

List of Abbreviations

Abbreviation	Meaning
ADA	American Diabetes Association
AGE	Advanced Glycation Products
BGLAP	Gene For Osteocalcin
BMD	Body Mass Density
cAMP	Cyclic Adenosine Monophosphate
cOC	Carboxylated Osteocalcin
CVD	Cardiovascular Disease
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
ELISA	Enzyme-Linked Immunoassay
FBG	Fasting Blood Glucose
FGF-23	Fibroblast Growth Factor -23
FSH	Follicle Stimulating Hormone
GDM	Gestational Diabetes Mellitus
GGCX	γ-glutamyl carboxylase
GH	Growth Hormone
GLP-1	Glucagon-Like Peptide-1
GnRH	Gonadotropin Releasing Hormone
GPRC6A	G Protein-Coupled Receptor Family C Group 6 Member A
HbA1c	Glycosylated Haemoglobin A1c
IGF-1	Insulin-Like Growth Factor 1
IR	Insulin Resistance
LH	Luteinizing Hormone
MODY	Maturity-Onset Diabetes Of The Young
mRNA	Messenger Ribonucleic Acid
OC	Osteocalcin
OGTT	Oral Glucose Tolerance Test
PRL	Prolactin
PTH	Parathyroid Hormone
RANKL	Receptor Activator Of Nuclear Factor-Kb Ligand
RER	Rough Endoplasmic Reticulum
SE	Standard Error
T1DM	Type 1 Diabetes Mellitus
T2DM	Type 2 Diabetes Mellitus

T3	Tri-Iodothyronine
T4	Thyroxin
TH	Thyroid Hormone
TNF-α	Tumor Necrosis Factor –A
TRs	Thyroid Hormone Receptors
TSH	Thyroid Stimulating Hormone
TT	Total Testesterone
ucOC	Undercarboxylated Osteocalcin
WHO	World Health Organisation

Chapter One Introduction

1. Introduction

Diabetes is a serious metabolic disease, which an increasing prevalence worldwide and the number of influenced individuals may reach to 693 million in 2045 of whom 85% will have type 2 diabetes (T2DM) (Cho *et al*., 2018; Basu *et al*., 2019).

Diabetes mellitus (DM) is a group of metabolic disorder diseases resulting from either defect in insulin secretion, insulin activity or both (WHO, 1999).

Diabetes mellitus can be accompanied by numerous complications including different organ systems such as skeleton. However, type 1 and type 2 diabetes mellitus affect bone differently; type 1 diabetes mellitus (T1DM), which mostly founds in young adults or adolescents, bone formation reduced and peak bone mass is poor (Hofbauer *et al*., 2007; Hamann *et al*., 2012). In contrast, type 2 diabetes mellitus (T2DM) have higher bone mineral density, but an increased risk of fractures because of impaired bone quality (Schwartz and Sellmeyer, 2004; Strotmeyer and Cauley, 2007).

Type 2 diabetes mellitus is described by insulin resistance in various insulin target cells, for example, myoblasts, hepatocytes and adipocytes (Shulman, 2000). T2DM, a complex multifactorial disease affect on sugar, fat and protein metabolism, it can lead to a dysregulation in the calcium, phosphorus and magnesium, thus leading to a series of complexities such as cardiovascular disease, neuropathy, retinal disease, and bone disease (Zakeri *et al.*, 2011; Asokan *et al.*, 2017).

Skeleton has long been considered as a target organ by T2DM, the hurtful effects of which on skeleton are multifactorial, modifying bone turnover, structure, strength and impaired bone quality compared to healthy controls (Carnevale *et al.*, 2014; Shanbhogue *et al.*, 2016).

The rate of bone turnover is reduced in patients with type 2 diabetes, as reflected by decreased expression of biomarkers of bone resorption and formation, including, calcium (Ca), parathyroid hormone (PTH) and osteocalcin (OC) (Gerdhem *et al.*, 2005).

Recently established that bone acts as an endocrine organ that secretes at least 3 hormones: fibroblast growth factor 23 (FGF23), lipocalin 2 (LCN2), and OC which suggest to modulates glucose metabolism, effect on fat deposition and blood sugar levels (Al-Rifai *et al.*, 2017).

Osteocalcin is the most abundant protein localciumted in the extracellular matrix of bone synthesized by osteoblasts (Zoch *et al.*, 2016). In humans, it is a 49 amino acid with three glutamic acid residues (Ferron and Lacombe, 2014).

Osteocalcin receptor G protein-coupled receptor family C group 6 member A (GPRC6A) is expressed in different tissues included the skeletal muscle, brain, testis, bone, liver and pancreatic β-Cells (Karsenty and Ferron, 2012).

Osteocalcin suggest to has numerous roles in deferent tissues, included effect in stimulate testosterone biosynthesis in Leydig cells, Glucose metabolism can be regulated by OC directly, throughout the receptor GPRC6A, increasing pancreatic β-cells proliferation improving insulin synthesis and secretion (Wei *et al.*, 2014) and indirectly by increasing

glucagon-like peptide-1 (GLP-1) secreted by intestinal enteroendocrine L-cells in the small intestine that has receptor on β-Cells enhancing insulin secretion (Lu *et al*., 2015). Insulin signaling in osteoblasts enhances OC action, which, in turn, favors insulin secretion (Shao *et al*., 2015).

Type 2 diabetes mellitus had been correlated with poor bone quality by several mechanisms involved, insulin resistance and hyperglycemia on the bone, accumulation advanced glycation end products effect on bone matrix proteins (Dhaliwal *et al* ., 2014; Starup-Linde and vestergaard, 2015).

1.1. Aim of the Study

This study is designed to evaluation and fulfill from relationship among osteocalcin (OC), insulin, reproductive hormones and some metabolic hormone in male with T2DM, by determent the following:

1. Fasting blood glucose (FBG) and Glycosylated haemoglobin A1c (HbA1c).
2. Levels of osteocalcin (OC) and insulin (IN) in the blood serum.
3. levels of reproductive hormones (Luteinizing hormone (LH), Follicle stimulating hormone (FSH), total testosterone (TT) and prolactin (PRL)
4. Levels of parathyroid hormone (PTH) and thyroid hormones (THs); (thyroid stimulating hormone (TSH), Tri-iodothyronine (T3) and Thyroxin (T4).
5. Estimate the total calcium (Ca) concentration in blood serum.

Chapter Two

Literature Reviews

2. Literature Review

2.1. Diabetes mellitus

Diabetes mellitus (DM) is a group of metabolic disorders in which there are high blood glucose levels over a prolonged period due to insufficient amount of insulin producing from pancreas or insulin resistance (Martin, 2006; Masharani and Michael, 2017).

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disorders of carbohydrate, fat and protein metabolism (Helrich, 1990). Several pathogenic processes are included in the development of diabetes; these range from autoimmune destruction of the β-cells of the pancreas with resultant insulin deficiency to abnormalities that result in resistance to insulin action (ADA, 2010).

Symptoms of marked hyperglycemia include; polyuria due to increased osmotic pressure, increased thirst sensation, increased fluid intake to try to compensate for increased urination, polydipsia, weight loss, although eating food freguently, poor healing of wounds, sometimes with polyphagia, and blurred vision (Powers, 2008).

There are many complications associated with the chronic hyperglycemia of diabetes including specific long term microvascular complexities affecting the kidneys, eyes, nerves, an increased risk for cardiovascular disease (CVD), diabetic ketoacidosis, and bone disease (Kitabchi *et al*., 2009; Punthakee *et al*., 2018).

Patients with T2DM have hyperinsulinemia, because of impaired cellular sensitivity to insulin, and hyperglycemia, because of insulin resistance (Yamaguchi and Sugimoto, 2011).

2.2. Classifications of Diabetes Mellitus

Classification of diabetes mellitus is established on its etiology and clinical presentation. And, there are four types or classes of diabetes mellitus; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types (ADA, 2013).

2.2.1. Type 1 Diabetes Mellitus (T1DM)

Type 1 diabetes mellitus (also named insulin dependent diabetes mellitus), this form which accounts for only 5-10% of DM patients (ADA, 2018). It is characterized by an deficiency of insulin level caused by an autoimmune attack on pancreatic β cells, leading to gradual reduction of the β cells population leads to deficiency of insulin secretion (Pugliese, and Eisenbarth, 2007; Bluestone *et al*.,2010).

Type 1 diabetes mellitus can affect children or adults, but traditionally was termed "juvenile diabetes" because a majority of this diabetes cases in the children (Rother, 2007).

2.2.2. Type 2 diabetes mellitus (T2DM)

Type 2 diabetes mellitus (also named non-insulin dependent diabetes or adult– onset diabetes mellitus) is more common than type 1, making up to 90% - 95% of DM cases (ADA, 2018), results from defects in the insulin action, altered cell receptors for insulin (insulin resistance) than deficiency (Atkinson

and Maciarnen, 1990). The risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (Shlomo, 2011). It is associated with lifestyle factors and genetics (Risérus *et al*., 2009). The majority of patients with this form are obese, which itself, causes insulin resistance (Lebovitz, 2004).

2.2.3. Gestational Diabetes Mellitus (GDM)

Gestational diabetes mellitus (GDM) making up to 3-20% of pregnancy, it's referring to glucose intolerance and recognition during pregnancy (ADA, 2019). GDM is a temporary type of diabetes that occurs during pregnancy most women with GDM will return to normal glucose levels after delivery (Smith-Morris, 2005).

Hormones and adipokines secreted from the placenta, comprising tumor necrosis factor (TNF)-α, human placental lactogen, and human placental growth hormone are possible causes of insulin resistance (IR) in pregnancy. In addition, increased progesterone, and cortisol during pregnancy play a role to a disruption of the glucose insulin balance (Barbour *et al.*, 2007).

To balance for the peripheral IR during pregnancy, insulin secretion increases from a woman's β-cell, the development of GDM happens when a woman's pancreas does not secrete sufficient insulin to keep up with the metabolic stress of the IR. In addition, increased maternal adipose deposition, decreased exercise, and increased caloric intake participate to this state of relative glucose intolerance (Alfadhli, 2015).

2.2.4. Other Types of Diabetes Mellitus

Monogenic diabetes is a rare disorder caused by genetic defects of pancreatic β-cell function results from inheritance of one or more mutations in a single gene causes β-cell dysfunction or insulin resistance (Amed and Oram, 2016; Misra and Hattersley, 2017). Monogenic diabetes consist of maturity-onset diabetes of the young (MODY) which is autosomal dominantly inherited diabetes that. In spite of a young age of onset (generally before age 25 yearss), its not insulin-dependent, results from β-cell dysfunction rather than insulin resistance and characterized by impaired insulin secretion with minimal or defects in insulin action (ADA, 2010). Another type of DM; neonatal diabetes; children diagnosed with diabetes within the first 6 months of life (referred to neonatal diabetes) are similar to have monogenic diabetes and not T1DM (ADA, 2016). These children commonly present with ketoacidosis and absent C-peptide (Guilar-Bryan and Bryan, 2008).

Other Specific types of diabetes mellitus due to other causes, such as diseases of the exocrine pancreas such as cystic fibrosis and pancreatitis, and drug or chemical induced diabetes (such as with glucocorticoid use, in the treatment of HIV/AIDS, or after organ transplantation) (ADA, 2019).

2.3. Diagnosis of Diabetes Mellitus

Diabetes Mellitus diagnosed according to (ADA, 2019) by the following.

- Fasting blood Glucose is ≥126 mg/dL (7.1 mmol/L), Fasting is defined as abstaining from food intake for at least 8 hours (usually overnight).
- Two-hour postprandial glucose ≥ 200mg/dL (11.1mmol/L) during oral glucose tolerance test (OGTT).
- HbA1c ≥ 6.5% (48mmol/mol) is also used in the diagnosis of diabetes.

- In a patients with classic symptoms of hyperglycaemia such as polyphagia, polydipsia and polyuria or hyperglycaemic, a random blood glucose ≥ 200 mg/dL (11.1 mmol/L) is diagnostic.

2.4.Complications of Diabetes Mellitus

2.4.1. Acute Complications

2.4.1.1. Diabetic ketoacidosis (DKA)

Diabetic ketoacidosis (DKA) is a common and life-threatening complication of type 1 diabetes. DKA is caused by very low levels of insulin and increased in counterregulatory hormones levels, such as glucagon, catecholamines, cortisol, and growth hormone (Wolfsdorf *et al*., 2014). This combination leads to catabolic changes in the metabolism of carbohydrates, fat, and protein, impaired glucose utilization and increased glucose production by the liver and kidneys result in hyperglycemia, lipolysis leads to increased production of ketones, especially beta-hydroxybutyrate (β-OHB), ketonemia, and metabolic acidosis (Kitabchi *et al.*, 2006).In type 2 diabetes patients, DKA occurs during concomitant acute illness or during transition to insulin dependency (Mauvais-Jarvis *et al*.,2004).

2.4.1.2. Hyperosmolar hyperglycemic state

The hyperosmolar hyperglycemic state is the most serious acute hyperglycemic emergency in patients with T2DM, it’s characterized by extreme elevations in serum glucose concentrations and hyperosmolality without ketosis (Pasquel and Umpierrez, 2014).These metabolic derangements result from synergistic factors including insulin deficiency and increased levels of counterregulatory hormones (glucagon, catecholamines, cortisol, and growth hormone) (Kitabchi *et al*., 2001). Hyperosmolar hyperglycemic state is associated with glycosuria, leading to osmotic diuresis, with loss of water,

sodium, potassium, and other electrolytes. In hyperosmolar hyperglycemic state, insulin levels are inadequate for glucose utilization by insulin sensitive tissues but sufficient (as determined by residual C-peptide) to prevent lipolysis and ketogenesis (ADA, 2001).

2.4.1.3. Hypoglycemia

Hypoglycemia is the most common, life-threatening acute complication of diabetes treatment; it's characterized by multiple risk factors and complex pathophysiology (Cryer, 2006). The brain depends on a continuous supply of glucose for energy, although it can also utilize ketone bodies. Young children and adolescents are at higher risk for hypoglycemia, and the spectrum of outcomes ranges from mild cognitive impairment to coma, seizure, and sudden death. The risk of hypoglycemia increases with duration of diabetes, partially due to progressive loss of alpha cell glucagon response to hypoglycemia, and is inversely related to preservation of beta cells (ADA, 2005).

2.4.2. Chronic Complications

2.4.2. 1. Diabetic Nephropathy

Diabetic nephropathy is a long-standing microvascular complication of diabetes and is the leading cause of end-stage renal disease (Chang *et al*., 2016). Nephropathy defens to damage of the glomerulus (filtering apparatus of the nephron) and capillaries associated with the glomerulus, leading to a reduction in the filtering capability of the kidneys (Černe, 2002).

2.4.2.2. Diabetic Retinopathy

Diabetic retinopathy is a specific fundus lesion that is the main cause of blindness in patients with diabetes (Flaxman *et al*., 2017) Based on the changes of haemodynamics or vascular geometry, vascular injury is considered to be

the prime motivator for the initiation and progression of diabetic retinopathy (Fletcher *et al*., 2007).

2.4.2.3. Diabetic Neuropathy

Diabetic neuropathy is one of the most common chronic complication of diabetes, characterized by damage to nerve glial cells, axons, and endothelial cells, and the morbidity from 30% to 50% in T2DM (Peltier *et al*., 2014). Diabetic peripheral neuropathy is the main clinical manifestation of sensory and autonomic nerve symptoms, distal symmetry polyneuropathy, and motor neuropathy are the most common types of diabetic peripheral neuropathy (Pop-Busui *et al*., 2017).

2.4.2.4. Cardiovascular Disease

The risk of cardiovascular disease (CVD) increased in diabetes, such that an individual with diabetes has a risk of myocardial infarction equivalent to that of nondiabetic individuals who have previously had a myocardial infarction (Haffner *et al*., 1998). High blood glucose levels stimulate the synthesis of advanced glycation end products, advanced oxidation protein products, and oxidation of low-density lipoprotein and that they are related to vascular injury in diabetes through several underlying processes that may be involved in the development and progression of atherosclerosis and could escalate the risk of CVD in patients with diabetes (Vamos *et al*., 2012)

2.5. Effects Diabetes on Bone

Diabetes Mellitus, especially T2DM, Its prevalence has increased along with the increase in obesity resultant from lifestyle changes of modern life (Moreira *et al*., 2015). Patients with T2DM, are at critical risk for well recognized diabetic complications, recently, one of more complications associated with DM, an increased risk of fragility fractures which seems to be

rather independent of bone mineral density (BMD) (Jackuliak and Payer, 2014; Liao *et al*., 2014). In general T2DM patients have normal BMD and abnormalities in bone material strength and/or bone microarchitecture (Farr and Khosla, 2016).

The mechanisms induced bone fragility in T1DM and T2DM were complex and partially overlap, patients with T1DM are mainly suffering from β-cell failure and low levels of Insulin-like growth factor-1 (IGF-1),which disrupt the function of osteoblasts during growth, so that low peak bone mass can occur at a young age (Hough *et al*.,2016; Napoli *et al*., 2017). In contrast, patients with T2DM developed bone fragility at a later stage of the disease, and consequently, at a later age due to the lack of insulin, advanced glycation end products (AGEs) accumulation on bone collagen, glucose toxicity, affect bone turnover (Napoli *et al*., 2014).

In general, the processes that included decreased bone formation in T2DM involve a decrease in bone quality, alterations of the mesenchymal cell differentiation and changes in osteoblasts and osteoclasts (Puspitasari *et al*., 2017).

Xu *et al*., (2014) suggest that bone turnover is impaired in T2DM, resulting in decreased osteoblastogenesis, osteoclastogenesis and bone mass.

2.6. Endocrine Mechanisms of Bone

skeleton is affected by various pathologic conditions, these being for example T1DM and T2DM or hypogonadism, such as the decline in estrogen during menopause, this may suggest, as proposed, that not only glucose metabolism and reproductive functions could have an influence on the skeleton, but that bone might interact with the respective organs to return a

signal, thereby keeping up a cross-talk among skeleton, pancreas, muscle, liver, and gonads (Schwetz, 2013).

The dominant paradigm in skeletal biology is that differentiation and functions of the two bone-specific cell types osteoblasts and osteoclasts, are determined by secreted molecules that can either be cytokines acting locally or hormones acting systemically (Harada and Rodan, 2003; Teitelbaum and Ross, 2003).

2.7. Action of Bone

Bone is a mineralized connective tissue that contains four types of cells: osteoblasts, bone lining cells, osteocytes, and osteoclasts (Downey and Siegel, 2006). Bone exerts numerous important roles in the body, such as locomotion, calcium and phosphate storage, support and protection of soft tissues, and harboring of bone marrow (Datta *et al*., 2008).

In spite of its inert appearance, bone is a highly dynamic organ that is continuously resorbed by osteoclasts and neoformed by osteoblasts (Bonewald, 2011). The role of bone lining cells suggest to plays an important role in coupling bone resorption to bone formation (Everts *et al*., 2002).

Normal bone remodeling is necessary for fracture healing and skeleton adaptation to mechanical use, as well as for calcium homeostasis, on the other hand, an imbalance of bone resorption and formation results in several bone diseases (Dallas *et al*., 2013).

In past 15 years they discovered that bone, as an organ, is not only a target of several endocrine signals, but itself acts as endocrine tissue. Indeed, bone cells are secreting at least two hormones, Fibroblast growth factor 23 (FGF23) and OC, which are involved in the regulation of phosphate homeostasis and

energy metabolism, respectively (Ferron, 2018). The exact role of FGF23 signaling in the parathyroid gland remains unclear since some studies found that it increases parathyroid hormone (PTH) secretion, while others found the opposite effect (Ben-Dov *et al*., 2007; Brownstein *et al*., 2008).

Osteocalcin is expressed and secreted by distinguished osteoblasts in bone and acts as a blood glucose-lowering hormone by stimulating insulin secretion by β-cells and by favoring insulin sensitivity in muscle, liver, and white adipose tissue (Pi *et al*., 2008).

2.8. Osteocalcin (OC)

OC also called "bone gamma-carboxyglutamic acid protein (BGP)" is the most abundant noncollagenous protein in bone matrix (Razzaque, 2011). It is a product of distinguished osteoblasts, formed by 46 to 50 amino acids related to species, in humans, it is a 49 amino acid (Villafan-Bernal *et al*., 2011). The human osteocalcin gene, BGLAP, is located on chromosome 1 at 1q25-q31 and encodes an 11kD, 98 amino acid pre-pro-protein (Zoch *et al*., 2016). The mature peptide is generated by sequential cleavage events that remove an endoplasmic reticulum signal sequence and the pro-sequence followed by γ-carboxylation of three glutamic acid residues at positions 17, 21, and 24 in human while at positions 13, 17 and 20 in mouse,(figure, 2-1) (Ferron and Lacombe, 2014).

OC found in two forms, undercarboxylated osteocalcin (ucOC) and fully carboxylated osteocalcin (cOC), cOC is formed after the glutamic acid residues of OC on the 17th, 21st, and 24th sites are carboxylated by vitamin K-dependent carboxylase, OC with noncarboxylated glutamic acid residues is known as ucOC (Pearson, 2007; Ferron, 2018).

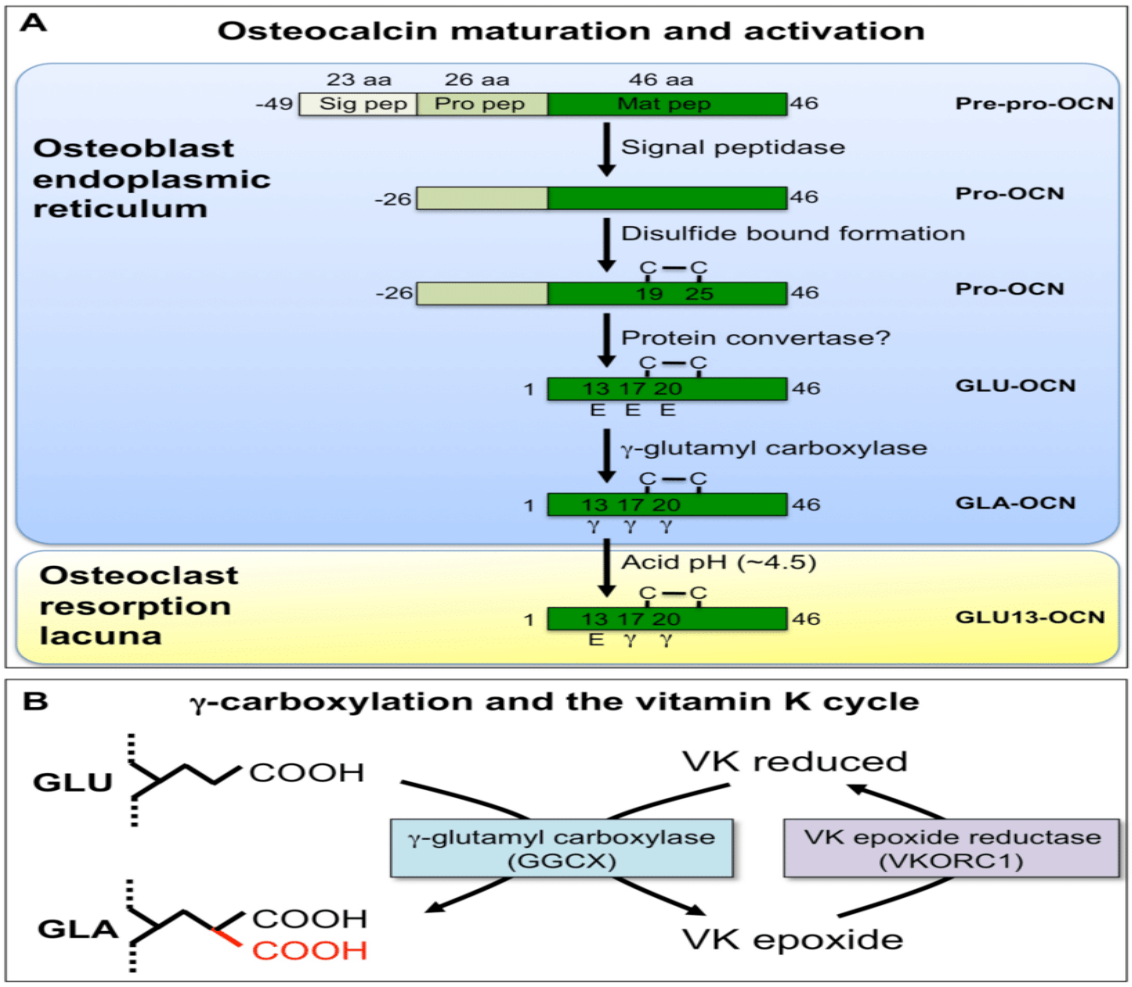


Figure (2-1): Osteocalcin maturation and activation steps (Ferron and Lacombe, 2014)

Only 10-30% of OC secreted reaches systemic circulation, while the remaining fraction is incorporated into bone matrix (Hernández-Gil, 2006). γ-Carboxylation of OC occurs in the RER and is mediated by γ-glutamyl calciumrboxylase (GGCX), which needs reduced vitamin K as an essential cofactor (Stafford, 2005). These posttranslational modification augments the affinity of OC for hydroxyapatite; the mineral component of bone extracellular matrix (ECM) (figure 2-2) (Hoang *et al*., 2003). Hence, the vast majority of

OC secreted by osteoblasts is trapped in bone ECM, in which it establishes the most abundant noncollagenous polypeptide (Ferron *et al*., 2010; Lacombe *et al*., 2013). Although both γ-carboxylated (Gla) and ucOC forms of OC are detected in serum, most *in vitro* and *in vivo* studies indicate that the endocrine function of OC is fulfilled by ucOC in mice and humans (Pi *et al*., 2011; Sabek *et al*., 2015; Bonneau *et al*., 2017).

Physiological functions of OC include maintaining normal bone mineralization, suppressing abnormal hydroxyapatite formation, and slowing down growth cartilage mineralization (Pearson, 2007). Besides its role in bone formation, studies suggest a potential relevance of undercarboxylated OC in energy metabolism and male fertility (Ducy, 2011; Karsenty, 2011; Oury, 2012). Pittas *et al*., (2009); Yeap *et al*., (2010) suggest there are correlations between total OC, including undercarboxylated and carboxylated OC, and fasting glucose or insulin. Im *et al*., (2008); Kindbom *et al*., (2009) found OC levels are lower in diabetic than in healthy subjects.

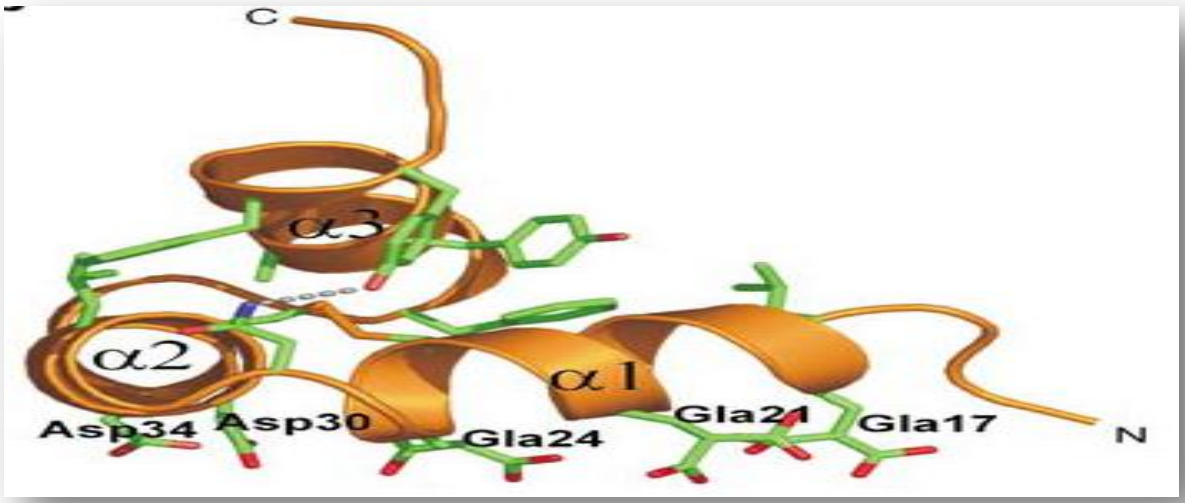


Figure (2-2): Three-dimensional structure of osteocalcin in huma (Hoang *et al*., 2003)

OC has receptor called G protein-coupled receptor (GPRC6A) is expressed in several tissues including the liver, muscle, skeletal, brain, testis, bone, and pancreatic β cells, figure (2-3) (Zoch *et al*., 2016).

Circulating OC levels vary according to age and gender (Midtby *et al*., 2001). The young adult male had higher levels than young women of the similar age (Fares *et al*., 2003; Jafari *et al.*, 2008). As they have longer and wider bones, and reach to peak BMD later in life (Szulc *et al*., 2007). After peak (BMD) is reached, serum OC levels decrease in women and male (Glover *et al*., 2008; Ardawi *et al*., 2010). In middle-aged and elderly male beyond 50–60 yearss of age, serum OC concentrations become stable or increase slightly (Gundberg *et al*., 2002).

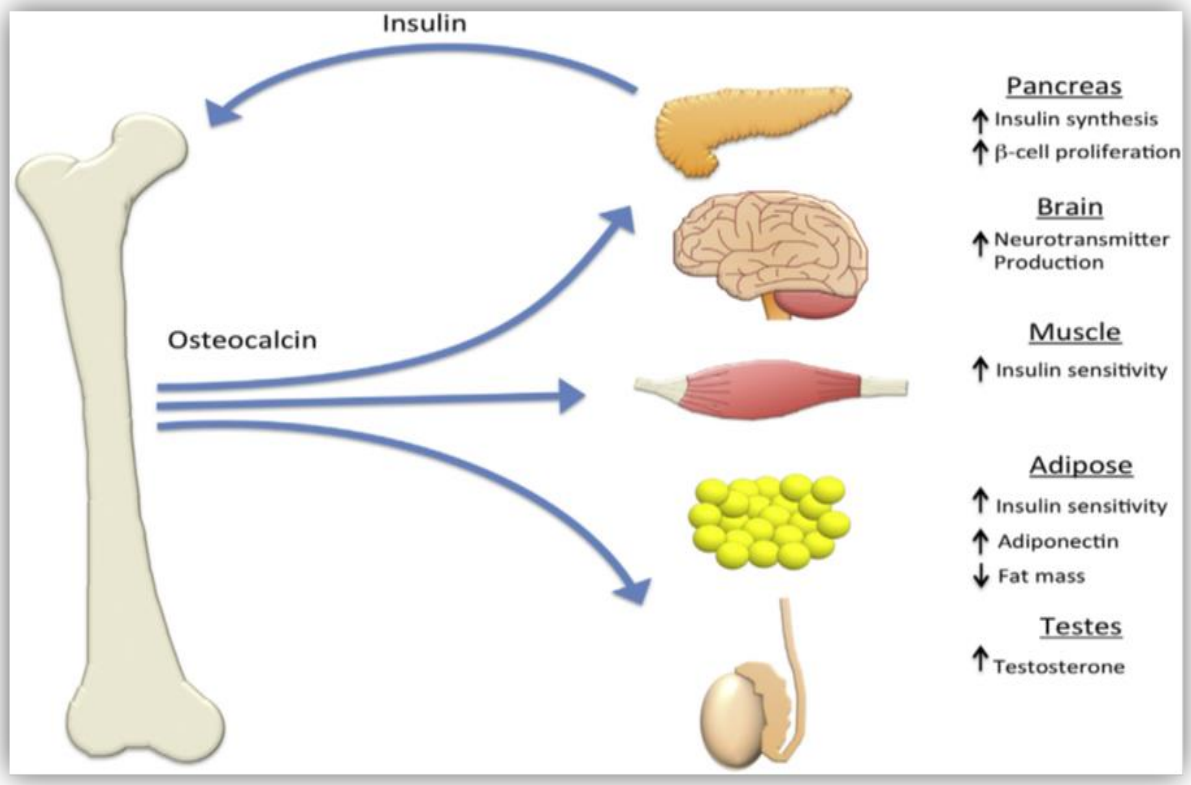


Figure (2-3): Effects of osteocalcin hormonal on whole body metabolis (Zoch *et al*., 2016)

OC is mainly synthesized by osteoblasts and is commonly used as a marker to denote the status of bone turnover, although its precise role within the bone matrix remains unclear (Zoch *et al*., 2016).

In animal models, unOC is suggested to increase insulin production and release by a direct effect on pancreatic β-cells (Zanatta *et al*., 2014). Conversely, insulin is believed to influence the osteoblasts directly and to augment OC production (Clemens and Karsenty, 2011).

Ferron and Lacombe, (2014) mentioned that in mice, the OC supports insulin secretion by pancreatic β cells and glucose utilization by peripheral tissues.

2.9. Function of Osteocalcin

2.9.1. Glucose Metabolism and Osteocalcin

The glucose metabolism regulation in mammals is a complex physiological process based on the interaction of a variety of hormones and other extracellular signals working on specific target tissues (Bilotta *et al.,* 2018). The major hormones involved in this process, insulin that plays a key role in promoting glucose uptake in muscle and adipose tissue, while it suppresses gluconeogenesis in the liver; Insulin thereby reducing postprandial glucose concentrations in the blood (Saltiel and Kahn, 2001).

Insulin receptor signaling pathway has been defined newly in the bone, precisely in osteoblasts (Ferron *et al.,* 2010). Harada and Rodan, (2003); Faienza *et al.,* (2015) found that bone can be a target of insulin action and a main regulator of energy metabolism by favoring β-cell insulin secretion and peripheral insulin sensitivity.

In specific, a major role in the glucose metabolism regulation, at this level, is attributed to osteoblasts that produce OC (Ng and Martin, 2009).

The evidence that OC may have an extraskeletal metabolic role beyond to its effect on bone is supported by data from mice lacking OC, in which an increase in visceral fat followed by hyperglycemia and hypoinsulinemia was observed, together with a decreased β-cell and insulin content (Ducy *et al*., 1996). Moreover promoting insulin secretion, OC increases peripheral insulin sensitivity (Kanazawa, 2015; Mizokami *et al.*, 2017).

Depending on experimental findings so far available, the presence of a positive loop between OC and insulin is suggested, by which OC stimulates insulin secretion, while the release of OC is enhanced by insulin, patients with insulin resistance have low circulating OC levels, and may have consequences in their bone structure and function (e.g., higher risk of fractures) thereby further strengthen the link between skeletal and glucose metabolism (Rubin, 2015).

In humans OC is inversely associated with FBG and HbA1c supporting the theory of a bone pancreas axis (Liu *et al*., 2015). Motyl *et al*., (2010) showed that poorly controlled blood glucose could lead to decreased serum OC level in diabetic patients, while serum OC increased after blood glucose was well controlled.

Lee *et al*., (2007) found that the changes of glucose metabolism could effect OC concentrations. OC stimulates insulin secretion by working directly on pancreatic β cells (Hinoi *et al*., 2008), and indirectly way through stimulation of glucagon-like peptide-1 (GLP-1) secretion by the gut (Mizokami *et al*., 2013).

Studies in mice revealed that OC function in β cells and myofibers is mediated through its binding to the GPRC6A (Pi *et al*., 2011; Mera *et al*., 2016). This pathway seems shows to be conserved in humans, since human OC can bind and activate human GPRC6A (De Toni *et al*., 2016).

2.9.2. Osteocalcin and Insulin Secretion

In vivo searches suggested that OC favors insulin secretion and have many independent biological effects on β-cells, which result altogether in a net increase in insulin secretion in response to glucose (Kanazawa, 2015). OC stimulates β-cell proliferation and thereby increases the β-cell pool, but it also promotes insulin synthesis and secretion directly, as well as indirectly way through GLP-1 (Kanazawa, 2015; Ferron, 2018). Insulin plays an anabolic effect on bones by stimulating osteoblast proliferation and differentiation (Yang *et al*., 2010).

Animal studies have demonstrated that diabetic rodents have diminished bone formation following bone injury, whereas insulin injection normalized it (Gandhi *et al*., 2005). Insulin deficiency, as in T1DM, is characterized by low quantities or activity of insulin-like growth factor-1 (IGF1) (Puspitasari *et al*., 2017). The stimulating activity of IGF1 on osteoblasts is inhibited by high level of AGEs or glucose (McCarthy *et al*., 2001) In contrast with T1DM, T2DM is a disease that generally shows insulin resistance, it remains unclear how in T2DM insulin resistance and insulin deficiency at later stage may influence bone turnover and fragility (Puspitasari *et al*., 2017).

Wei *et al*., (2014) suggest that the OC stimulates insulin secretion and production from pancreatic β-cells through two distinct signaling pathways; First, OC stimulates directly, within minutes insulin secretion by β-cells, and

second, OC stimulates within a few hours the expression at the mRNA levels of the two genes encoding insulin in mice, Ins1 and Ins2. Both of these effects are reliant on the presence of a functional GPRC6A at the surface of pancreatic β-cells, since OC is unable to stimulate insulin secretion or insulin gene expression in pancreatic islets lacking GPRC6A (Pi *et al*., 2011; Wei *et al*., 2014).

Mechanistically, the critical effect of OC on insulin secretion appears to be mediated through the generation of cAMP in the cells subsequent activation of GPRC6A, this increased cAMP levels in turn may stimulate an increase in the cytosolic content of Calcium which is known to support the release of insulin granules from β-cells (Hinoi *et al*., 2008).

There is evidence that OC supports insulin secretion indirectly through glucagon-like peptide-1 (GLP-1). GLP-1 is the one of the incretin hormones, is produced by enteroendocrine L cells of the small intestine and secreted into the circulation in response to nutrient ingestion (Drucker, 2006). GLP-1 promotes insulin secretion by pancreatic β-cells in conditions of high glucose levels (Mizokami *et al*., 2013).

Mizokami *et al*., (2014) and Pi *et al.,* (2016) found that when orally managed, the osteoblast derived hormone OC has the ability to indirectly control insulin secretion, not only through production of other insulin-regulating hormones, such as testosterone, but also by prompt of intestinal GLP-1 secretion by OC receptor GPRC6A was detected in intestinal endocrine cells expressing GLP-1. GLP-1 has been revealed to regulate both bone remodeling and energy homeostasis (Diamanti-Kandarakis *et al*., 2011).

2.9.3. Reproductive Hormone and Osteocalcin

The testis function regulation is classically ascribed to the hypothalamic pituitary gonadal axis. According to this endocrine pathway, the hypothalamic release of gonadotropin releasing hormone (GnRH) promotes the production of the two gonadotropins, namely, luteinizing hormone (LH) and, follicle-stimulating hormone (FSH) by the adenohypophysis, following, FSH and LH act on the testis by promoting, respectively, the spermatogenesis and the production of testosterone (TT) that in turn, inhibits both GnRH an gonadotropins release with a negative feedback loop (Du Plessis *et al*., 2010).

Leydig cells of the testis are responsible for producing TT, which in turn regulates the development of male reproductive organs, maturation of germinal cells, and production of estrogen by aromatization. The major physiological regulator of TT production by Leydig cells is the LH (Burns *et al*., 2002).

In male, TT plays a key role in stimulating secondary sexual characteristics and maintaining the function of male reproductive tissues such as the testis and prostate, in addition, it is necessary for health (Spitzer *et al.*, 2013) as well as, favors bone growth, maturation, and maintenance and prevention of osteo-porosis (Mosekilde *et al*., 2013; Shao *et al*., 2015). El and Azar, (2013) remember decline TT level in the patients withT2DM.

Ducy *et al*., (1996) revealed the existence of a parallel endocrine pathway acting on the endocrine function of the testis, which involves a OC. OC effects on the TT concentration through promote it is production by the testes (Oury *et al*., 2011). In clinical practice, there was also a positive relationship between TT and OC (Kanazawa *et al*., 2013).

The study of Oury *et al*., (2011) on male mice found that OC regulate the synthesis of TT by Leydig cells, while TT is also reported to modulate the osteoblastic expression of OC in male rats (Steffens *et al*., 2014).

The OC stimulates TT biosynthesis in the mouse testis by binding to GPRC6A in Leydig cells, interestingly; OC-deficient mice exhibit increased concentrations of LH (Di Nisio *et al.*, 2017). Furthermore, there are growing evidence that OC concentrations are a dependable marker of insulin secretion and sensitivity and circulating concentrations of TT in humans, by using mouse models, found that the OC and LH act in 2 parallel pathways and that OC-stimulated TT synthesis is positively controlled by bone resorption and insulin signaling in osteoblasts (Oury *et al*., 2013; Karsenty and Oury, 2014).

2.9.4. Osteocalcin and Thyroid Hormones

Thyroid gland is endocrine gland in the body located in the anterior triangle of neck in front of thyroid cartilage. The thyroid gland secretes two biologically active thyroid hormones (THs); thyroxine (T4) and Tri-iodothyronine (T3) (Kopp, 2005). TH synthesis and secretion is regulated by a negative-feedback system that includes the hypothalamus, pituitary, and thyroid gland (the HPT axis). Thyroid stimulating hormone (TSH) that secretion from pituitary is the principal regulator of THs release and secretion; it has a critical role in thyroid growth and development (Fekete and Lechan, 2014).

Thyroid hormones are essential for normal skeletal development, maturation and metabolism; hypothyroidism weakens skeletal maturation, resulting in growth retardation and skeletal abnormalities (Allain and

McGregor, 1993). A key osteoblast protein revealed to be regulated by thyroid hormone by increased OC mRNA half-life (Gouveia *et al*., 2001).

The THs receptor modulates bone turnover, like to the steroid hormone receptor superfamily; hyperthyroidism is a major cause of secondary osteoporosis (Khosla and Melton, 1995). The receptor for T3 founds on osteoblasts, and the THs reportedly promote the secretion of OC in osteoblasts (Stern, 1996).

The biological functions of the thyroid hormone are mainly mediated by binding to specific receptors in the nucleus, and that the receptor-hormone complex activates the transcription of related genes (Mullur *et al*., 2014). Kainuma *et al.*, (2016) reported that the T3 stimulated OC synthesis in osteoblasts through transcriptional levels. However, the exact mechanism underlying the thyroid hormone-induced synthesis of osteocalcin remains to be elucidated.

Thyroid hormones may act on bone cells indirectly by increasing secretion of growth hormone (GH) and IGF-1, or directly by affecting target genes via particular nuclear receptors. The presence of thyroid hormone receptors (TRs) has been showed in human and rodent osteoblast-like cells and cell lines and newly in osteoclasts derived from an osteoclastoma *in vitro* (Abu *et al*., 1997).

2.9.5. Osteocalcin and Parathyroid Hormone

Parathyroid hormone (PTH) is a polypeptide produced by the parathyroid glands that keeps Calcium homeostasis, because of it’s stimulatory effects on osteoclastic resorption and on the renal reresorption of Calcium, it shows both anabolic and catabolic effects, depending in whether it is managed intermittently or continuously respectively (Massaro and Rogers, 2004).

Declined bone remodeling in T2DM has been demonstrated by a number of lines of evidence, levels of PTH tend to be 20%–50% lower in the T2DM subjects than in the controls, and even in the setting of reduced (Dobnig *et al.*, 2006; Yamamot *et al.*, 2012; Ardawi *et al.*, 2013).

Parathyroid Hormone binds to its specific cell membrane receptor and activates several signal transduction pathways, resulting in increased BGLAP gene expression to OC mRNA that is then translated in the rough endoplasmic reticulum (RER) in to OC (Yu *et al*., 2008).

D'Amelio *et al.*, (2015) reported, postmenopausal osteoporotic females treated with PTH have increased circulating OC and unOC levels and decrease blood glucose concentrations during an oral glucose tolerance test. However, in hypoparathyroid patients treated with PTH cause an increase in unOC but do not affect fasting plasma glucose or insulin resistance (Harslof *et al.*, 2015)**.**

Osteocalcin embedded in bone matrix must be released during bone resorption, the amount of OC increased in the presence of PTH; a stimulator of resorption (Ivaska *et al.*, 2004).

In mineral homeostasis, low level of circulating Calcium promotes the parathyroid gland to release PTH, which later up regulates blood Calcium levels by stimulating osteoclastic bone resorption, renal Calciumreresorption, and renal production of vitamin D to increase intestinal Calcium resorption. Increased serum phosphate and vitamin D stimulate FGF23 synthesis and releasing in bone, which subsequently inhibits PTH production from the parathyroid gland, inhibits vitamin D production in the kidney, and stimulates renal phosphate excretion (Figure, 2-2) (Shao *et al*., 2015).

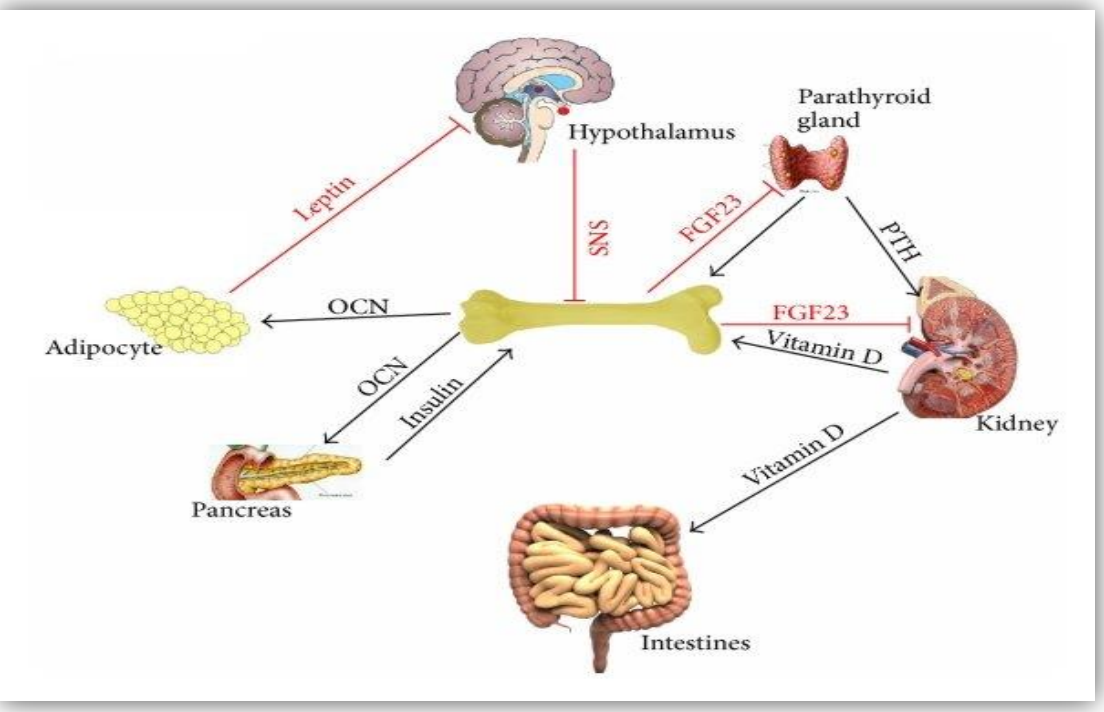


Figure (2-3): Skeleton regulates mineral and energy homeostasis (Shao *et al*., 2015).

2.10. Glycated Hemoglobin A1c (HbA1c)

HbA1c is glycated hemoglobin in which glucose is attached to the N terminal valine residue of each b-chain of hemoglobin A (HbA1c). Since the life span of erythrocytes is; 120 days, HbA1c reflects the average glucose concentration over the preceding 8–12 weeks (Goldstein *et al*.,2004). It is formed in a non-enzymatic pathway by hemoglobin's normal exposure to high plasma levels of glucose. The use of this test has been extended to diagnose and screen for diabetes mellitus with the endorsement of several international diabetes societies and the WHO. In 2010, the International Expert Committee and the American Diabetes Association proposed diagnostic criteria for diabetes and pre-diabetes based on HbA1c levels. These are HbA1c ≥6.5% to

diagnose diabetes mellitus and between 5.7–6.4% for pre-diabetes (ADA, 2013). The glycated hemoglobin has been used as a biomarker of long-term glycemic control (Goldenburg and Punthakee, 2013). HbA1c values between 6% - 7% were considered as a sign of good control of diabetes, HbA1c values between 7.1% -8% indicated moderated control, and HbA1c value >8% were designated as poor control of diabetes (Marshall, 2010).

HbA1c is considered the golden standard in the management of type 2 diabetes mellitus (T2DM). It is part of the glycemic objectives and mentioned in all the guidelines and clinical recommendations for the management of T2DM, also increased HbA1c values are associated with increased risk of T2DM specific complications (ADA, 2013).

Chapter Three

Material and Methods

3. Material and methods

3.1. Material

3.1.1. Apparatus and Equipment

The apparatus and equipment used in this study are summarized in table (3-1)

Table (3-1): The apparatus and equipment that used with their producing companies and countries

NO.	Apparatus and Equipment	Company	origin
1	Alcohol methyl	Meheco	China
2	Centrifuge	Eppendorf AG	Germany
3	Cobas e411	Roche	Germany
4	Cobas integra 400 plus	Roche	Germany
5	Cotton	Kardelen	Turkey
6	Dual-program (D-10)	Bio- Rad	Italy
7	Disposable syringe 10mL	TROJECTOR-3	Germany
8	EDTA tube	AFCO	Jordan
9	ELISA reader	Bio-Tek	Germany
10	ELISA washer	Bio-Tek	Germany
11	Gel tube	Sun	Jordan
12	Incubator	Heraeus	Germany
13	Plain tube	AFMA	Jordan
14	Refrigerator	Concord	Lebanon

3.1.2. Kits

The kits used in this study are summarized in table (3-2)

Table (3-2): The kits that used with their producing companies and countries

No.	Kit	Company	origin
1	Follicle stimulating hormone(FSH)	Mannheim	Germany
2	Glucose	Mannheim	Germany
3	Insulin	Demeditec	Germany
4	Luteinizing hormone (LH)	Mannheim	Germany
5	Osteocalcin (OC)	Shanghai	China
6	Parathyroid hormone (PTH)	Mannheim	Germany
7	Prolactin (PRL)	Mannheim	Germany
8	Testosterone(TT)	Mannheim	Germany
9	Thyroid-stimulating hormone (TSH)	Mannheim	Germany
10	Thyroxine (T4)	Mannheim	Germany
11	Total Calcium(Ca)	Mannheim	Germany
12	Triiodothyronine (T3)	Mannheim	Germany

3.2. Subjects of the Study

This study was carried out at the center for Endocrinology and diabetes specialist in Misan province during the period from December of 2018 to May of 2019.

The population of this study consists of 110 male with average aged (35-49) years of whom 66 type 2 diabetic (uncontroling) and 44 healths male.The 44 healthy male represent the control group. The researcher excluded some of the patients because of the history of cardiopathy, nephropathy, hypertension, smoking, bone disease and thyroid gland disease (Appendix1)

3.3. Experimental Design

The study population included 110 male (66 diabetic and 44 healthy as control group), ranging in age from 35 to 49 years. The study population divided by age into three groups, the first (35-39) years , second (40-44) years and third (45-49) years, also divided by the body mass index into three groups, normal weight (18.5-24.9 kg/m^2), overweight (25-29.9 kg/m^2), obesity (BMI > 30 kg/m^2) according (WHO,2019), figure (3-1):

BMI calculated by dividing the weight (kg) by height square (m2) according to: BMI= weight (kg) /height² (m2).

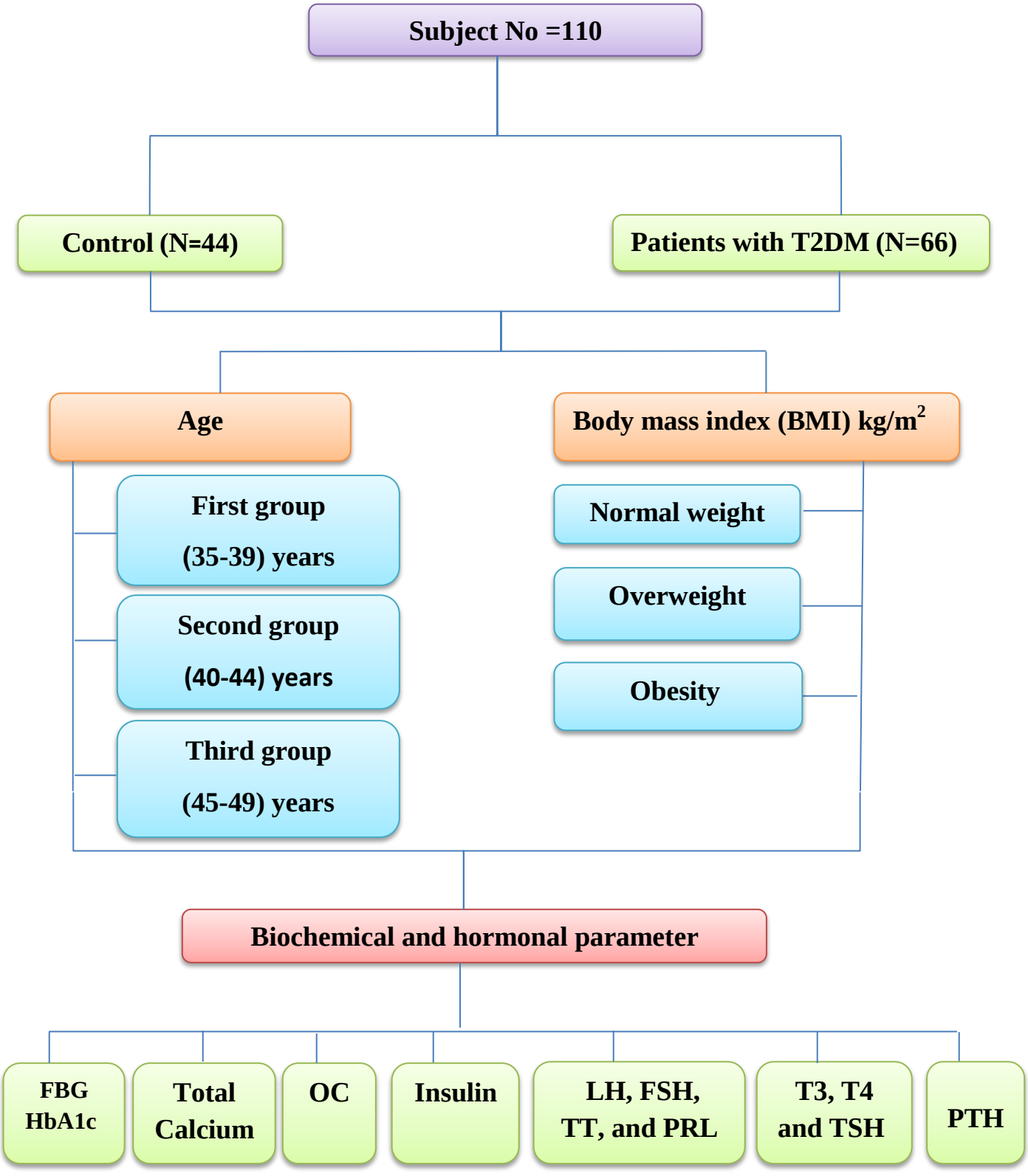


Figure (3-1): Experimental Design

3.4. Blood Collection

Venous blood Sample (8-10 mL) was drowning from each fasting patient and control at (8-10 AM). The blood sample was divided into fractions; 2 mL was transferred into EDTA tube for HbA1c determination, and other fraction of blood sample was left for 15 minutes to clot at room temperature ,and then serum (2 - 2.5 mL) was separated by centrifugation at 3000 (rpm) for 15 (min), to measure glucose and total Calcium levels. Last serum was transferred into labeled plain tube and stored at -20 Ċ° until used for evaluation of hormones (OC, Insulin, LH, FSH, TT, PRL, T3, T4, TSH and PTH).

3.5. Methods

3.5.1. Biochemical Parameters

3.5.1.1. Determination of HbA1c

Principle of Procedure

The D-10 Dual program is based on chromatographic separation of the analytes by ion –exchange high –performance liquid chromatographic (HPLC). The sample is automatically diluted on the D-10 and injected into the analytical cartridge.

The D-10 delivers a programmed buffer gradient of increasing ionic strength to the cartridge, where the hemoglobin is separated based on their ionic interaction with the cartridge material. The separated hemoglobin then passes through the flow cell of the filter photometer, where change in the absorbance at 415 nm is measured. A calibrator is analyzed with each run

for adjustment of the calculation parameters for determination of HbA1c; the value recorded is in percent according to (Chandrashekar, 2016).

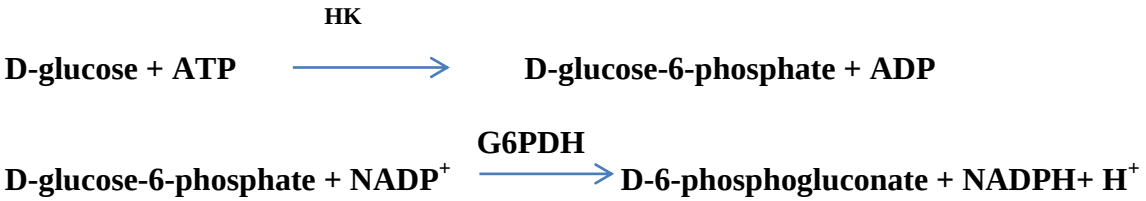
Procedure

- The HbA1c kit put out of refrigerator for 10 min
- The reagents are placed in their place on the side of the external device.
- 2 mL of blood was taken by medical syringe and place it in EDTA tube and then, the sample mixed gently by inverting the tube.
- The sample tubes allowed reaching the room temperature (25 c) before performing assay.
- The sample tube is loaded into the D-10 sample rack and put it in the place known inside the device D-10
- Patient QC ID was appearing on the screen after they have been acanned by the barcode reader
- The DONE button was press after you have entered each patient ID
- The START button was press to begin the analysis
- The steps for the device followed to start the callibration process automatically.

3.5.1.2. Fasting Blood Glucose Measurement

Test Principle

Hexokinase (HK) catalyzes the phosphorylation of glucose by ATP to form glucose-6-phosphate and ADP. To follow the reaction, a second enzyme, glucose-6-phosphate dehydrogenase (G6PDH) is used to catalyze oxidation of glucose-6-phosphate by NADP+ to form NADPH (Tietz, 2006).



The concentration of the NADPH formed is directly proportional to the glucose concentration. It is determined by Cobas integra 400 plus systems automatically.

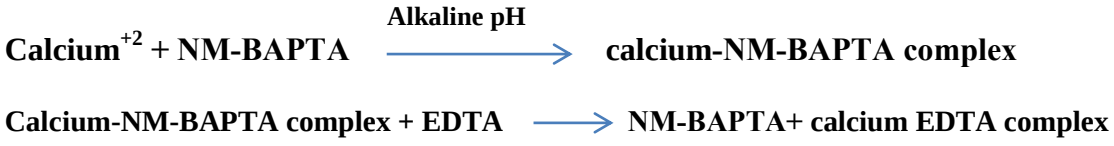
Normal values FBG for male

4.11-6.05 mmol/L (74-109 mg/dL)

3.5.1.3. Determination of Calcium

Test Principle

Calcium ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA. (Endres and Rude, 2006).



The change in absorbance is directly proportional to the calcium concentration and is measured photometrically by Cobas integra.

Cobas integra 400 plus systems automatically calculate the analyte concentration of each sample.

Normal values F.B.G for male

8.6 mg/dI -10.3 mg/dI.

3.5.2. Determination of Hormones

3.5.2.1 Determination of Osteocalcin

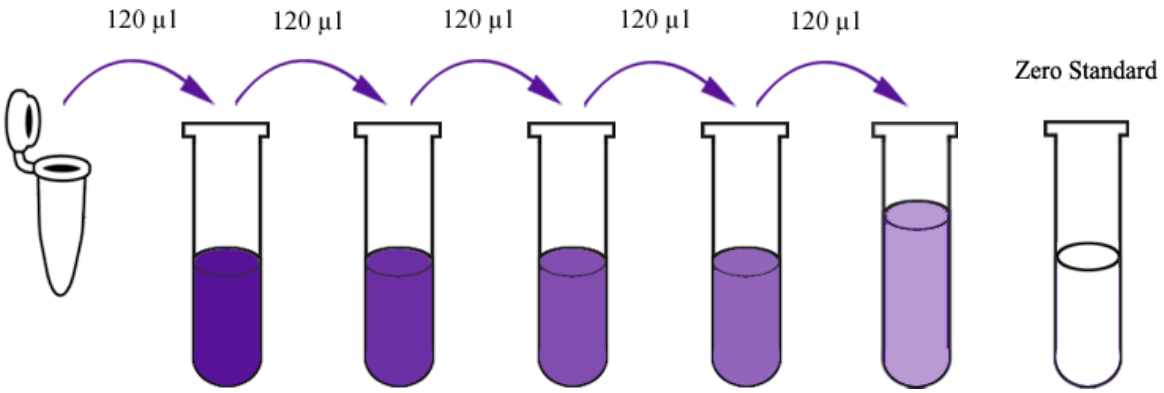
Assay Principle

Serum osteocalcin determined by kit supply from (Shanghai Company, China). This kit uses Enzyme-Linked Immunosorbent Assay (ELISA) (Power and Fottrell, 1991).

Reagent Preparation

- All reagents should be brought to room temperature before use.
- **Standard** Reconstitute the 120 µl of the standard (160 ng/mL) with 120 µl of standard diluent to generate a 80 ng/mL standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (80 ng/mL) 1:2 with standard diluent to produce 40 ng/mL, 20 ng/mL, 10 ng/mL and 5 ng/mL solutions. Standard diluent serves as the zero standard (0 ng/mL). Dilution of standard solutions suggested are as follows:

80ng/mL	Standard No.5	120µl Original Standard + 120µl Standard Diluent
40ng/mL	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
20ng/mL	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
10ng/mL	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
5ng/mL	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
160ng/mL	80ng/mL	40ng/mL	20ng/mL	10ng/mL	5ng/mL

- **Wash Buffer** Dilute 20mL of Wash Buffer Concentrate 30x into deionized or distilled water to yield 500 mL of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Assay Procedure

1. Prepare all reagents, standard solutions and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature.
2. Determine the number of strips required for the assay. Insert the strips in the frames for use. The unused strips should be stored at 2-8°C.
3. Add 50µl standard to standard well
4. Add 40µl sample to sample wells and then add 10µl anti-OT/BGP

antibody to sample wells, and then add 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5. Remove the sealer and wash the plate 5 times with wash buffer. Soak wells with at least 0.35 mL wash buffer for 30 seconds to 1 minute for each wash. For automend washing, aspirate all wells and wash 5 times with wash buffer, overfilling wells with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. Add 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Add 50µl Stop Solution to each well, the blue color will change into yellow immediately.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution (Appendix 2).

Normal values for male

0.5 ng/mL – 150 ng/mL

3.5.2.2. Determination of Insulin

Principle of Method

The Demeditec INS-EASIA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on microtiterplates. The assay uses monoclonal antibodies (MAbs) directed against distinct epitopes of insulin. Callibrators and samples react with the capture monoclonal antibody

(MAb1) coated on microtiter well and with a monoclonal antibody (MAb 2) labelled with horseradish peroxidase (HRP). After an incubation period allowing the formation of a sandwich: coated MAb 1 – human insulin – MAb 2 – HRP, the microtiterplate is washed to remove unbound enzyme labelled antibody. Bound enzyme-labelled antibody is measured through a chromogenic reaction. Chromogenic solution (TMB ready for use) is added and incubated. The reaction is stopped with the addition of Stop Solution and the microtiterplate is then read at the appropriate wavelength. The amount of substrate turnover is determined colourimetrically by measuring the absorbance, which is proportional to the insulin concentration (Starr *et al.*, 1978).

Procedure

1. Select the required number of strips for the run.

2. Secure the strips into the holding frame.

3. Add 50 µl of each Callibrator, Control and Sample into the appropriate wells.

4. Add 50 µl of anti-INS-HRP conjugate into all the wells.

5. Incubate for 30 minutes at room temperature on a horizontal shaker set at 700 rpm ± 100 rpm.

6. Aspirate the liquid from each well.

7. Wash the plate 3 times by:

_ dispensing 0.4 mL of Wash Solution into each well

_ aspirating the content of each well

8. Add 200 µl of the freshly prepared revelation solution into each well within 15 minutes.

Following the washing step.

9. Incubate the microtiterplate for 15 minutes at room temperature on a horizontal shaker set at 700rpm ± 100 rpm, avoid direct sunlight.

10. Add 50 µl of Stop Reagent into each well.

11. Read the absorbances at 450 nm and 490 nm (reference filter 630 nm or 650 nm) within 1 hour and calculate the results (Appendix 3)

Normal values for male

2 µIU/mL to 25 µIU/ mL

3.5.2.3. Determination of Reproductive, Thyroid and Parathyroid Hormones

Assay Principle

The Elecsys assay combines conventional antigen–antibody reactions on the surface of a streptavidin-coated paramagnetic microparticle with electrochemical reaction on the surface of an electrode, which generates luminescence (Figure, 3-2) (Wild, 2013).

- A biotin-labeled antibody and a ruthenium-labeled antibody are incubated with the sample analyte. An antigen or nucleic acid probe may be substituted for either antibody to accommodate other assay types.
- The immune complex is captured by the streptavidincoated microparticles, which have a high biotin-binding capacity.

- Inside the ECL measuring cell, the microparticles with their bound immune complexes are uniformLy deposited on the electrode. Unbound components are washed away.
- The sample analyte is quantitated by applying a voltage to the electrode and measuring the ECL signal.
- Once the ECL reaction is completed, the magnetic microparticles are released from the surface and washed away. The surface is thoroughly cleaned, and the cell is ready for another measurement (Figure, 3-3) (Wild, 2013).

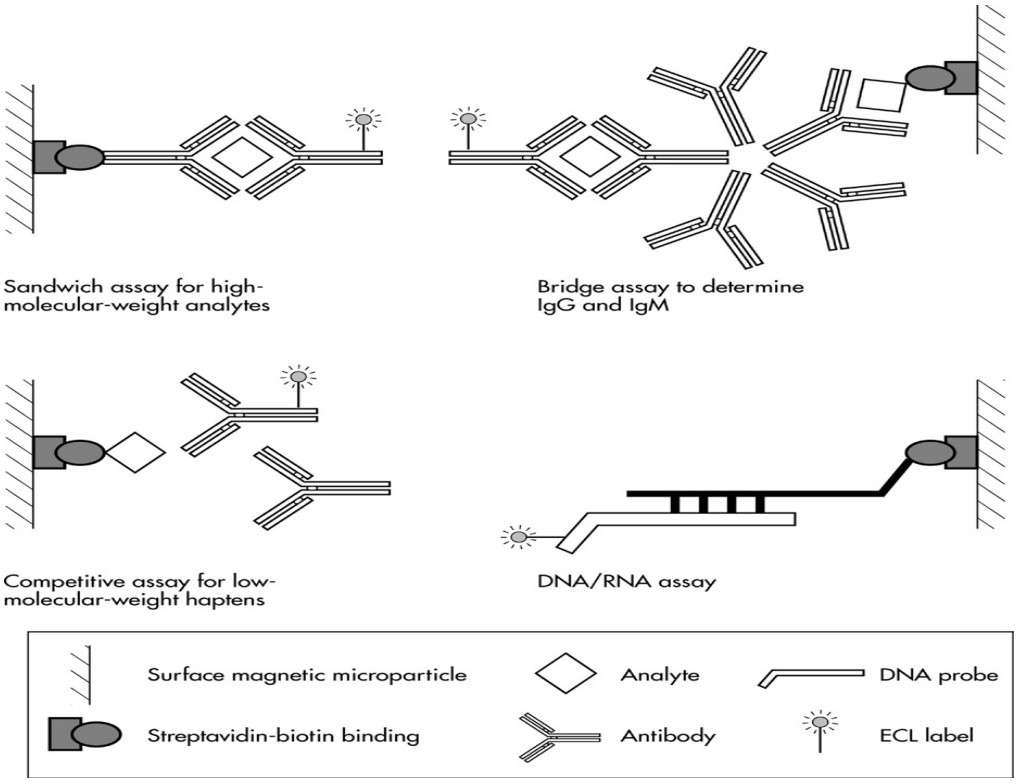


Figure (3-2) Elecsys assay formats

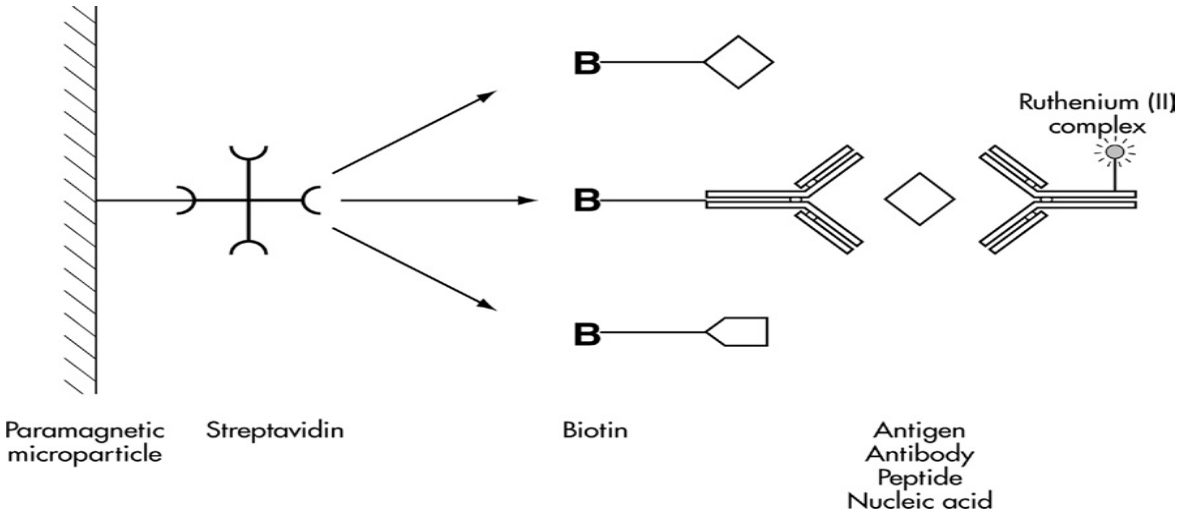


Figure (3-3): Elecsys assay principle

Preparation of instrument

1- Load assay cups and assay tips in consumables
2- Regents of each parameter (LH,FSH,TT, PRL,T3,T4,TSH and PTH), loading in reagent rock in the instrument (cobas e411)
3- Make callibration and control of each reagent
4- Load the sample of serum in to sample rack then the instrument automatically began analysis as following:

3.5.2.3.1. Luteinizing Hormone (LH) Concentration

Principle Test:

Sandwich principle (the Elecsys LH assay employs two monoclonal antibodies specifically directed against human LH (Tietz, 1995).

Procedure:

- 1st incubation: 20 µL of sample, a biotinylated monoclonal LH-specific antibody, and a monoclonal LH-specific antibody labeled with a

ruthenium complex a (Tris (2, 2'-bipyridyl) ruthenium (II)-complex (Ru (bpy) form sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal values for male

1.7 m.lu/mL -7 m.lu/mL

3.5.2.3.1. Follicle Stimulating Hormone (FSH) Concentration

Sandwich principle (the Elecsys FSH assay employs two different monoclonal antibodies specifically directed against human FSH) (Wu, 2006).

Procedure:

- 1st incubation: 40 µL of sample, a biotinylated monoclonal FSH-specific antibody, and a monoclonal FSH-specific antibody labeled with a ruthenium complex a (Tris (2, 2'-bipyridyl) ruthenium (II)-complex (Ru (bpy) form a sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal Values for Male

1.7 m.lu/mL -12 m.lu/mL

3.5.2.3.3. Testosterone (TT) Concentration

Competition principle (The elecsys testosterone II assay is based on a competitive test principle using a high affinity monoclonal antibody specifically directed against testosterone) (Rosner *et al.*, 2006).

Procedure:

- 1st incubation: 20 µL of sample is incubated with a biotinylated monoclonal testosterone-specific antibody. The binding sites of the labeled antibody become occupied by the sample analyte (depending on its concentration).

- 2nd incubation: After addition of streptavidin-coated microparticles and a testosterone derivate labeled with a ruthenium complex a (Tris (2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy), the complex becomes bound to the solid phase via interaction of biotin and streptavidin.

- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal Values for Male

2.80 ng/mL- 8 ng/mL

3.5.2.3.4. Prolactin (PRL) Concentration

Sandwich principle (the Elecsys Prolactin II assay uses two monoclonal antibodies specifically directed against human prolactin) (Fahie-Wilson *et al.*, 2000).

Procedure:

- 1st incubation: 10 µL of sample and a biotinylated monoclonal prolactin specific antibody form a first complex.
- 2nd incubation: After addition of a monoclonal prolactin-specific antibody labeled with a ruthenium complex a (Tris (2, 2'-bipyridyl) ruthenium (II)-complex (Ru (bpy) and streptavidin-coated microparticles, a sandwich complex is formed and becomes bound to the solid phase via interaction of biotin and streptavidin
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M.

Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal Values for Male

4.6 ng/mL- 21 ng/mL

3.5.2.3.5. Tri-iodothyronine (T3) Concentration

Competition principle (the Elecsys T3 assay employs a competitive test principle with polyclonal antibodies specifically directed against T3) (Wild, 1994).

Procedure:

• 1st incubation: 30 µl of sample and the specific antibodies anti-T3, labeled with a ruthenium complex; bound T3 is released of the binding proteins in the sample by ANS.

• 2nd incubation: After the incorporation of the coated microparticles of streptavidin and of biotinylated T3, the attachment sites still free of the labeled antibody are occupied, with the formation of an antibody-hapten complex. The complex formed binds to the by the interaction of biotin and streptavidin.

• The reaction mixture is aspirated into the reading cell, where the microparticles are magnetically attached to the surface of the electrode. Unconnected elements are then removed with ProCell. The application of an electric current to the electrode induces a chemiluminescent which is measured by a photomultiplier.

Normal Values for Male

1.3 nmol/L-3.1 nmol/L

3.5.2.3.6. Thyroxine (T4) Concentration

Competition principle (the Elecsys T4 assay employs a competitive test principle with an antibody specifically directed against T4) (Wu, 2006).

Procedure:

- 1st incubation: 15 µL of sample and a T4-specific antibody labeled with a ruthenium complex; bound T4 is released from binding proteins in the sample by ANS (8-anilino-1-naphthalene sulfonic acid).
- 2nd incubation: After addition of streptavidin-coated microparticles and biotinylated T4, the still-free binding sites of the labeled antibody become occupied, with formation of an antibody-hapten complex. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Applicaltion of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal Values for Male
66 nmol/L-181 nmol/L

3.5.2.3.7. Thyroid Stimulating Hormone (TSH) Concentration

Test principle

Sandwich principle (The Elecsys TSH assay employs monoclonal antibodies specifically directed against human TSH) (Wu, 2006).

Procedure:

- 1st incubation: 50 µL of sample, a biotinylated monoclonal TSH-specific antibody and a monoclonal TSH-specific antibody labeled with a ruthenium complex react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal Values for Male
0.270-4.20 µIU/mL

3.5.2.3.8. Parathyroid Hormone (PTH) Concentration

Elecsys assay for determining intact PTH employs a sandwich test principle in which a biotinylated monoclonal antibody reacts with the N-terminal fragment (1-37) and a monoclonal antibody labeled with a ruthenium complex(a) reacts with the C-terminal fragment (38-84) (Ohe *et al*., 2003).

Procedure:

- 1st incubation: 50 µL of sample, a biotinylated monoclonal PTH-specific antibody, and monoclonal PTH-specific antibody labeled with a

ruthenium complex a (Tris (2, 2'-bipyridyl) ruthenium (II)-complex (Ru (bpy) sandwich complex.

- 2nd incubation: After addition of streptavidin-coated microparticles, the complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

Normal Values for Male

15-65 pg/mL (1.6-6.9 pmol/L) (Thomas, 1998).

3.6. Statistical Analysis

Data were analyzed by T.test and one way ANOVA by general liner model procedure using SPSS version 23 statistic program .the comparisons between means were made using least significant differences (LSD) using Genstat3statistic program .The difference were considered to be significant at $P<0.05$ using multivariate model in SPSS. The data are presented as mean ± S.E (standard error). The correlation coefficient Pearson was calculated to examine association among parameters (SPSS, 2015).

Chapter Four

The Results

4. Results

4.1. Comparison of biochemical and hormonal parameters between diabetic mellitus patients and control groups

The values FBG and HbA1c increase significantly (P<0.05) in patients groups (207.57±8.66 mg/dI) and (9.39±0.28%) respectively in comparison with control groups (93.90±1.58 mg/dI) and (5.27±0.04%) respectively, (Table, 4-1).

The values of total Calcium don't different significantly (P<0.05) in comparison between patients (9.51±0.12 mg/dI) group and control (9.31±0.11 mg/dI), (Table, 4-1).

The OC and insulin values increase significantly (P<0.05) in patients groups (1.53±0.15 ng/mL) and (20.07±1.96 μIU/mL) respectively, compare with control groups (0.85±0.11 ng/mL) and (13.88±0.80 μIU/mL) respectively, (Table, 4-1).

The values of LH, FSH and TT don't different significantly (P<0.05) in comparison between patients groups (5.86±0.26), (5.36±0.37) and (3.49±0.17 ng/mL) respectively and control groups (5.58±0.34), (4.56±0.29) and (3.29±0.14 ng/mL) respectively, while the PRL values decrease significantly (P<0.05) in patients (9.54±0.53 ng/mL) group compare with control (11.38±0.74 ng/mL) group, (Table, 4-1).

The values of T3, T4 and TSH don't different significantly (P<0.05) in comparison between patients groups (1.77±0.04 nmol/L), (98.40±2.27 nmol/L) and (1.65±0.09 μIU/mL) respectively and control groups (1.88±0.04 nmol/L), (98.03±2.67 nmol/L) and (1.75±0.1 μIU/mL 4) respectively, (Table, 4-1).

The values of PTH don't different significantly (P<0.05) in comparison between patients (5.18±0.26 pg/mL) group and control (4.98±0.21 pg/mL) group, (Table, 4-1).

Table (4-1): biochemical and hormonal parameters between T2DM patients and control groups (mean ± S.E)

Parameters		control	patients	P-value
		n=44	n=66	
FBG (mg/dI)		93.90±1.58	207.57±8.66 *	0.00
HbA1c (%)		5.27±0.04%	9.39±0.28*	0.00
Total Calcium (mg/dI)		9.31±0.11	9.51±0.12	0.25
OC (ng/mL)		0.85±0.11	1.53±0.15 *	0.00
IN(µIU/mL)		13.88±0.80	20.07±1.96*	0.00
Reproductive hormones	LH (m.lu/mL)	5.58±0.34	5.86±0.26	0.51
	FSH (m.lu/mL)	4.56±0.29	5.36±0.37	0.09
	TT (ng/mL)	3.29±0.14	3.49±0.17	0.39
	PRL (ng/mL)	11.38±0.74	9.54±0.53*	0.04
Thyroid hornones	T3 (nmol/L)	1.88±0.04	1.77±0.04	0.07
	T4 (nmol/L)	98.03±2.67	98.40±2.27	0.91
	TSH (µIU/mL)	1.75±0.14	1.65±0.09	0.59
PTH (pg/mL)		4.98±0.21	5.18±0.26	0.56

*significant between control and patients at the p<0.05)

4.2. Biochemical Analysis (According to Age)

4.2.1. Fasting Blood Glucose and Glycosylated hemoglobin A1c

The value of FBG in patients in first (210.61±14.96 mg/dI), second (207.09±15.44 mg/dI) and third (205.03±15.29 mg/dI) age groups increased significantly (P<0.05) comparison with the first (94.67±2.37 mg/dI), second (93.05±2.42 mg/dI) and third (93.90±3.31 mg/dI) control groups respectively (Table, 4-2).

The results showed no significant (P<0.05) differences in FBG value among patients of T2DM in first, second and third age groups (Table, 4-2).

The value HbA1c of patients in first (9.27±0.47%), second (9.55±0.45%) and third (9.45±0.54%) groups increased significantly (P <0.05) comparison with the control in first (5.09±0.08%), second (5.35±0.05%) and third (5.39±0.04%) groups respectively (Table, 4-2).The results showed no significant differences in HbA1c value among patients of T2DM in first, second and third groups (Table, 4-2).

Table (4-2): The values of FBG and HbA1c in control and patients with T2DM (According to Age) (mean ± S.E)

Groups	Parameters	Control	Patients	P-value
First group (35-39)years	**FBG (mg/dI)**	(n=16)	(n=22)	0.00
		94.67±2.37	210.61±14.96* a	
	HbA1c %	5.09±0.08	9.27±0.47* a	0.00
Second group (40-44)years	**FBG (mg/dI)**	(n=14)	(n=22)	0.00
		93.05±2.42	207.09±15.44* a	
	HbA1c%	5.35±0.05	9.55±0.45* a	0.00
Third group (45-49)years	**FBG (mg/dI)**	(n=14)	(n=22)	
		93.90±3.31	205.03±15.29* a	0.00
	HbA1c%	5.39±0.04	9.45±0.54*a	0.00

*significant between control and patients at the (p<0.05)

Similar letters refer to non- significant differences among patients groups

4.2.2. Total Calcium (Ca)

The value of total Calcium in patients of second group (9.81±0.27 mg/dI) increases significantly (P <0.05) comparison with the second control group (8.99±0.12 mg/dI); there are no significant differences in Calcium value patients of first (9.36±0.16 mg/dI) and third (9.33±0.18 mg/dI) as compared with control of first (9.34±0.22 mg/dI) and third (9.59±0.18 mg/dI) groups respectively (Table, 4-3).

The results showed no significant differences in total Calcium value among patients of T2DM in first, second and third groups (Table, 4-3)

Table (4-3): Total Ca values in control and patients with T2DM (According to Age) (mean± S.E.)

Groups	Parameter	Control	Patients	P-value
First group (35-39)years	**Ca (mg/dI)**	(n=16)	(n=22)	0.95
		9.34±0.22	9.36±0.16 [a]	
Second group (40-44)years	**Ca (mg/dI)**	(n=14)	(n=22)	0.01
		8.99±0.12	9.81±0.27[*] [a]	
Third group (45-49)years	**Ca (mg/dl)**	(n=14)	(n=22)	0.32
		9.59±0.18	9.33±0.18 [a]	

*Significant between control and patients at the (p<0.05)

Similar letters refer to non- significant differences among patients groups

4.3. Hormonal Parameters (According to Age)

4.3.1. Osteocalcin Level

The OC level in patients of second (2.01±0.31ng/mL) and third (1.83±0.31 ng/mL) groups increase significantly (P<0.05) in comparison with the control of second (0.97±0.22 ng/mL) and third (0.55±0.18 ng/mL) groups. There are no significant differences in OC level between patients (1.03±0.21ng/mL) and control (1.08±0.15ng/mL) of first group. The results showed that the OC level among patients with T2DM increased significantly (P<0.05) in second group in comparison with first group, while there are no significant difference between second and third groups (Table, 4-4).

The results showed no significant difference between third and first groups of T2DM (Table, 4-4).

4.3.2. Insulin Level

The level of insulin in patients of T2DM in first (22.45±4.40 μIU/mL) group increases significantly (P <0.05) in comparison with the control of first (10.90±1.03 μIU/mL) group. There are no significant difference of insulin levels in patients of second (18.98±2.74 μIU/mL) and third (19.23±2.90 μIU/mL) comparison to the control of second (15.38±1.79 μIU/mL) groups and third (15.79±3.40 μIU/mL) group.

The results showed no significant difference in insulin level among patients of T2DM in first, second and third groups (Table, 4-4).

Table (4-4): The values of OC and insulin in control and patients with T2DM (According to Age) (mean ± S.E)

Groups	Parameters	Control	Patients	P-value
First group (35-39) years	OC ng/mL	(n=16)	(n=22)	0.84
		1.08±0.15	1.03±0.21 [b]	
	IN μIU/mL	10.90±1.03	22.45±4.40 [*a]	0.01
Second group (40-44) years	OC ng/mL	(n=14)	(n=22)	0.01
		0.97±0.22	2.01±0.31 [*a]	
	IN μIU/mL	15.38±1.79	18.98±2.74 [a]	0.28
Third group (45-49) years	OC ng/mL	(n=14)	(n=22)	0.01
		0.55±0.18	1.83±0.31 [*ab]	
	IN μIU/mL	15.79±3.40	19.23±2.90 [a]	0.26
LSD	(OC = 0.98)			

*significant at the p<0.05) between control and patients

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.3.3. Reproductive Hormones

Table (4-5) shows that there are no significant differences (p<0.05) in the level of LH in patients of the first group (5.00±0.31 m.lu/mL) and second group (6.13±052 m.lu/mL) when compared to the control in first (5.22±0.55 m.lu/mL) and second group (6.65±0.68) respectively. While the level of LH in patients of third group (6.62±2.34 m.lu/mL) increase significantly (p<0.05) when compared with the control of third group (4.90±0.41 m.lu/mL) (Table, 4-5).

The results of current study indicate increased the LH level among patients of T2DM significantly (p<0.05) in third group compared with the patients of first group. There are no significant differences (p<0.05) between third and second groups, also there are no significant different between second and first groups (Table, 4-5).

There are no significant differences ($p<0.05$) in the values of FSH in patients of first (4.33±0.53 m.lu/mL), second (6.20±0.76 m.lu/mL) and third (5.95±0.58 m.lu/mL) groups as comparison with the control in the first (4.24±0.31 m.lu/mL), second (4.63±0.52 m.lu/mL) and third (4.85±0.67 m.lu/mL) groups respectively (Table, 4-5).

The results showed no significant differences ($p<0.05$) in FSH values among patients of T2DM in all patients age groups (Table, 4-5).

The same table (4-5) showed the values of TT showed no significantly differences ($P <0.05$) in the patients of first (2.86±1.45 ng/mL), second (3.64±0.20 ng/mL) and third (3.89±0.29 ng/mL) groups when compared with control of first (3.05±0.26 ng/mL), second (3.39±0.19 ng/mL) and third (3.47±0.26 ng/mL) groups respectively.

The results showed the value of TT among patients of T2DM which increased significantly ($P <0.05$) in third group as comparison with the first group.

There are no significant differences ($p<0.05$) between third and second group, also there are no significant differences ($p<0.05$) between first and second groups (Table, 4-5).

The value of PRL in patients of first group (9.40±0.93 ng/mL) decrease significantly ($P>0.05$) in comparison with first control group (13.01±1.36 ng/mL).There are no significant differences ($p<0.05$) in patients of second group (9.94±0.80 ng/mL) and third group (8.56±0.88 ng/mL) as compared with control of second (11.08±1.49 ng/mL) and third (9.82±0.76 ng/mL) groups, (Table, 4-5).

The same table showed no significant differences ($p<0.05$) in PRL value among all patients groups.

Table (4-5): The values of reproductive hormones in control and patients with T2DM (According to Age) (mean ± S.E)

Groups	Parameters	Control	Patients	P-value
First group (35-39)years	LH (m.lu/mL)	(n=16) 5.22±0.55	(n=22) 5.00±0.31 [b]	0.71
	FSH (m.lu/mL)	4.24±0.31	4.33±0.53 [a]	0.89
	TT (ng/mL)	3.05±0.26	2.86±1.45 [b]	0.64
	PRL (ng/mL)	13.01±1.36	9.40±0.93 [* a]	0.03
Second group (40-44)years	LH (m.lu/mL)	(n=14) 6.65±0.68	(n=22) 6.13±052 [ab]	0.54
	FSH (m.lu/mL)	4.63±0.52	6.20±0.76 [a]	0.09
	TT (ng/mL)	3.39±0.19	3.64±0.20 [ab]	0.38
	PRL (ng/mL)	11.08±1.49	9.94±0.80 [a]	0.50
Third group (45-49)years	LH (m.lu/mL)	(n=14) 4.90±0.41	(n=22) 6.62±2.34 [*a]	0.01
	FSH (m.lu/mL)	4.85±0.67	5.95±0.58 [a]	0.23
	TT (ng/mL)	3.47±0.26	3.89±0.29 [a]	0.30
	PRL(ng/mL)	9.82±0.76	8.56±0.88 [a]	0.32
LSD	(LH =1.62) , (TT =1.03)			

*significant between control and patients at the ($p<0.05$)

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.3.4. Thyroid Hormones

As shown from table (4-6) there are no significant differences of T3 values in patients in first group (1.76±0.07 nmol/L), second group (1.78±0.08 nmol/L) and third group (1.79±0.06 nmol/L) when we compared to the control in first (1.86±0.07 nmol/L), second (1.85±0.03 nmol/L) and third (1.94±0.09 nmol/L) groups respectively.

The same table showed no significant ($P < 0.05$) difference in T3 value among patients of T2DM in first, second and third groups

Table (4-6) shown there are no significant differences of T4 values in all patients of first (99.91±4.00 nmol/L), second (96.90±3.60 nmol/L) and third (94.53±3.60 nmol/L) groups when compared with the control of first (95.64±4.80 nmol/L), second (96.87±2.19 nmol/L) and third (101.94±5.78 nmol/L) groups respectively.

The results showed no significant difference in T4 value among patients of T2DM in first, second and third groups (Table, 4-6).

Table (4-6) showed the value of TSH no significant different ($P < 0.05$) in patients of the first (1.53±0.13 μIU/mL), second (1.46±0.13 μIU/mL) and third (1.87±0.15 μIU/mL) groups when compared to control of first (2.13±0.30 μIU/mL), second (1.24±0.18 μIU/mL) and third (1.83±0.18 μIU/mL) groups.

The results showed the value of TSH in patients of T2DM which increase significantly ($P < 0.05$) in third group comparison with second group. There are no significant difference ($P < 0.05$) between patients of first and third groups, also no significant difference ($P<0.05$) between patients of first group in comparison with the second group (Table, 4-6).

Table (4-6): The values of thyroid hormones in control and patients with T2DM (According to Age) (mean ± S.E)

Groups	Parameters	Control	Patients	P-value
group First (35-39)years	T3 nmol/L	(n=16) 1.86±0.07	(n=22) 1.76±0.07 [a]	0.36
	T4 nmol/L	95.64±4.80	99.91±4.00 [a]	0.5
	TSH µIU/mL	2.13±0.30	1.53±0.13 [ab]	0.08
Second group (40-44)years	T3 nmol/L	(n=14) 1.85±0.03	(n=22) 1.78±0.08 [a]	0.45
	T4 nmol/L	96.87±2.19	96.90±3.60 [a]	0.99
	TSH µIU/mL	1.24±0.18	1.46±0.13 [b]	0.33
Third group (45-49)years	T3 nmol/L	(n=14) 1.94±0.09	(n=22) 1.79±0.06 [a]	0.16
	T4 nmol/L	101.94±5.78	94.53±3.60 [a]	0.28
	TSH µIU/mL	1.83±0.18	1.87±0.15 [a]	0.85
LSD	(TSH= 0.41)			

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.3.5. Parathyroid Hormone Level

As show in table (4-7), the PTH value decrease significantly ($p>0.05$) in patients of first (4.65±0.26 pg/mL) group when compare it with first control (5.62±0.32 pg/mL) group. There are no significant differences ($p<0.05$) in the PTH level in patient of second (5.07±0.42 pg/mL) and third (5.76±0.60 pg/mL) groups when compare with control of second (4.36±0.27 pg/mL) and third (4.87±0.42 pg/mL) groups respectively.

The results showed no significant differences among patients of T2DM in first, second and third age groups, (Table 4-7).

Table (4-7): The value of PTH in control and patients with T2DM (According to Age) (mean± S.E.)

Groups	Parameter	Control	Patients	P-value
First group (35-39)years	PTH pg/mL	(n=16) 5.62±0.32	(n=22) 4.65±0.26*a	0.02
Second group (40-44)years	PTH pg/mL	(n=16) 4.36±0.27	(n=22) 5.07±0.42 a	0.17
Third group (45-49)years	PTH pg/mL	(n=16) 4.87±0.42	(n=22) 5.76±0.60 a	0.23

*significant between control and patients at the ($p<0.05$)

Similar letters refer to non- significant differences among patients groups

4.4. Biochemical Analysis (According to Body Mass Index)

4.4.1. Fasting Blood Glucose (FBG) Level and Glycosylated hemoglobin A1c (HbA1c)

The FBG value increase significantly ($P<0.05$) in the patients of normal weight (205.40±26.34 mg/dI), overweight (219.63±11.34mg/dI) and obesity (186.95±14.82 mg/dI) groups as comparison with the control groups in normal weight (103.48±3.31mg/dI), overweight (87.71±2.26 mg/dI), obesity (87.71±2.26 mg/dI) groups respectively, (Table, 4-8).

The results showed no significant difference ($P<0.05$) in FBG values among patients of T2DM in different BMI groups (Table, 4-8).

The HbA1c level of patients in normal weight (9.05±0.71%), overweight (9.78±0.39%) and obesity (186.95±14.82%) groups increased significantly ($P<0.05$) as comparison with the control groups of normal weight (5.32±0.03%), overweight (5.33±0.05%) and obesity (5.2020±0.08%) groups respectively (Table, 4-8).

The results showed no significant difference in HbA1c value among patients of T2DM in different BMI groups (Table, 4-8).

Table (4-8): The values of FBG and HbA1c in control and patients T2DM (According to BMI) (mean±S.E)

Groups	Parameters	Control	Patients	P-value
Normal weight (18.5-24.9)	FBG(mg/dI)	(n=8) 103.48±3.31	(n=10) 205.40±26.34$^{*\ a}$	0.04
	HbA1c %	5.32±0.03	9.05±0.71$^{*\ a}$	0.01
Overweight (25-29.9)	FBG(mg/dI)	(n=16) 87.71±2.26	(n=36) 219.63±11.34$^{*\ a}$	0.00
	HbA1c %	5.33±0.05	9.78±0.39$^{*\ a}$	0.00
Obesity (BMI>30)	FBG(mg/dI)	(n=20) 95.02±2.00	(n=20) 186.95±14.82$^{*\ a}$	0.00
	HbA1c %	5.2020±0.08	8.8710±0.47^{*a}	0.00

*significant between control and patients at the ($p<0.05$)

Similar letters refer to non- significant differences among patients groups

4.4.2. Total Calcium (Ca)

Calcium level in the patients of overweight (9.67±0.19mg/dI) group increase significantly ($P<0.05$) in comparison with the overweight control (9.07±0.15mg/dI) group, there are no significant differences in patients of normal weight (9.50±0.15) and obesity (9.21±0.18mg/dI) when compare them with the control of normal weight (9.85±0.34mg/dI) and obesity (9.29±0.15mg/dI) respectively (Table, 4-9).

The results showed there are no significant differences in Calcium level among patients of T2DM in different BMI groups (Table, 4-9)

Table (4-9): The value of Calcium in control and patients with T2DM (According to BMI) (mean± S.E.)

Groups	Parameter	Control	Patients	P-value
Normal weight (18.5-24.9)	Ca (mg/dI)	(n=8) 9.85±0.34	(n=10) 9.50±0.15 [a]	0.38
Overweight (25-29.9)	Ca (mg/dI)	(n=16) 9.07±0.15	(n=36) 9.67±0.19[* a]	0.02
Obesity (BMI>30)	Ca (mg/dI)	(n=20) 9.29±0.15	(n=20) 9.21±0.18[a]	0.746

*significant between control and patients at the ($p<0.05$)

Similar letters refer to non- significant differences among patients groups

4.5. Hormonal Parameters (according to body mass)

4.5.1. Osteocalcin Level

The OC value increased significantly ($P <0.05$) in patients of overweight (1.77±0.24 ng/mL) and obesity (1.32±0.22 ng/mL) groups compared to the control of overweight (1.00±0.23 ng/mL) and obesity (0.71±0.15 ng/mL) groups. While no significant ($P <0.05$) differences in OC value in patients of normal weight (1.07±0.25 ng/mL) group compared to the control of normal weight groups (0.89±0.19ng/mL), (Table, 4-10).

The results showed no significant difference in OC value among patients of T2DM in different BMI groups (Table, 4-10).

4.5.2. Insulin

As shown in table (4-10) the value of insulin in patients of normal weight (14.24±1.99 µIU/mL) and obesity (27.35±5.06µIU/mL) groups increase

significantly (P <0.05) when compare them with the control of normal weight (8.14±0.29 μIU/mL) and obesity (13.10±0.79μIU/mL) groups respectively. While no significant difference (P <0.05) in insulin value in patients of overweight (17.64±1.97 μIU/mL) groups compared to the control of overweight (17.73±1.39 μIU/mL) group.The results showed the value of insulin in patients of obesity which increase significantly (P <0.05) in comparison with the normal weight and overweight groups, While no significant difference between patients of normal weight and overweight groups, (Table, 4-10).

Table (4-10): The values of OC and insulin in control and patients with T2DM (according to BMI) (mean± S.E.)

Groups	Parameters	Control	Patients	P-value
Normal weight (18.5-24.9)	OC(ng/mL)	(n=8) 0.89±0.19	(n=10) 1.07±0.25 [a]	0.57
	IN μIU/mL	8.14±0.29	14.24±1.99[*b]	0.01
Overweight (25-29.9)	OC ng/mL	(n=16) 1.00±0.23	(n=36) 1.77±0.24[* a]	0.02
	IN μIU/mL	17.73±1.39	17.64±1.97[b]	0.96
Obesity (BMI>30)	OC ng/mL	(n=20) 0.71±0.15	(n=20) 1.32±0.22[* a]	0.03
	IN μIU/mL	13.10±0.79	27.35±5.06[*a]	0.01
LSD	(IN=9.71)			

*significant between control and patients at the (p<0.05)

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.5.3. Reproductive Hormones

The LH value of patients in overweight (5.90±0.40 m.lu/mL) group increased significantly (P <0.05) in comparison with the control of overweight group (4.63±1.04 m.lu/mL). There are no significant differences (p<0.05) in the value of LH in patients of normal weight (6.51±0.49 m.lu/mL) and obesity

(5.48±0.44m.lu/mL) groups compared with control of the normal weight (7.62±1.19 m.lu/mL) and obesity (5.51±0.45 m.lu/mL) groups.The results showed no significant differences (P <0.05) in LH value among patients of T2DM in different BMI groups (Table, 4-11).

As show in table (4-11), there are no significant differences (p<0.05) in the value of FSH in patients of normal weight (6.13±1.43 m.lu/mL), overweight (5.46±0.45 m.lu/mL) and obesity (4.81±0.63m.lu/mL) as compared to control of normal weight (4.09±0.76 m.lu/mL), overweight (4.40±0.43 m.lu/mL) and obesity (4.88±0.45 m.lu/mL) groups respectively (Table, 4-11).The results showed no significant differences (P <0.05) in FSH value among patients of T2DM in different BMI groups (Table, 4-11).

The results show TT value of patients no different significantly (P <0.05) in normal weight (3.64±0.27 ng/mL), overweight (3.59±0.26 ng/mL), and obesity (3.23±0.33ng/mL) when compared with control of normal weight (3.39±0.14 ng/mL), overweight (3.16±0.20 ng/mL), and obesity (3.36±0.26ng/mL) groups respectively. There no significant differences (P <0.05) in TT value among patients of T2DM in different BMI groups.

As shown in table (4-11), the value of PRL in patients of normal weight patients (7.35±1.08 ng/mL) decrease significantly (P<0.05) in comparison with the control of normal weight (15.50±2.39 ng/mL) group. There are no significant differences (p<0.05) in patients of overweight (9.33±0.58 ng/mL) and obesity (11.03±1.26[a] ng/mL) when compared with control of overweight (9.42±0.72) and obesity (11.30±1.02 ng/mL) groups respectively.

The results showed PRL value in patients of obesity increase significantly (P <0.05) in compared with patients of normal weight. While no significant

differences ($p<0.05$) between patients of normal weight and overweight, also no significant differences ($p<0.05$) in comparison between patients of overweight and obesity groups (Table, 4-11).

Table (4-11): The values of reproductive hormones in control and patients with T2DM (according to BMI) (mean± S.E)

Groups	Parameters	Control	Patients	P-value
Normal weight (18.5-24.9)	LH m.lu/mL	(n=8)	(n=10)	0.41
		7.62±1.19	6.51±0.49	
	FSH m.lu/mL	4.09±0.76	6.13±1.43	0.42
	TT ng/mL	3.39±0.14	3.64±0.27	0.42
	PRL ng/mL	15.50±2.39	7.35±1.08^{*b}	0.01
Overweight (25-29.9)	LH m.lu/mL	(n=16)	(n=36)	0.01
		4.63±1.04	5.90±0.40*	
	FSH m.lu/mL	4.40±0.43	5.46±0.45	0.10
	TT ng/mL	3.16±0.20	3.59±0.26	0.19
	PRL ng/mL	9.42±0.72	9.33±0.58^{ab}	0.91
Obesity (BMI>30)	LH m.lu/mL	(n=20)	(n=20)	0.952
		5.51±0.45	5.48±0.44	
	FSH m.lu/mL	4.88±0.45	4.81±0.63	0.92
	TT ng/mL	3.36±0.26	3.23±0.33	0.757
	PRL ng/mL	11.30±1.02	11.03±1.26^{a}	0.873
LSD	(PRL= 4.33)			

*significant between control and patients at the ($p<0.05$)

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.5.4. Thyroid Hormones

As shown table (4-12) There are no significant differences (P <0.05) of T3 values in patients of normal weight (1.91±0.11 nmol/L) overweight (1.77±0.06 nmol/L) and obesity (1.71±.07nmol/L) when compared with control of normal weight (1.95±0.05 nmol/L), overweight (1.82±0.03 nmol/L) and obesity (1.91±0.08nmol/L) groups respectively.

The results showed there are no significant differences (P <0.05) in T3 value among patients of T2DM in different BMI groups (Table, 4-12).

Same table shown there are no significant differences (P <0.05) of T4 values in patients of normal weight (101.53±5.59 nmol/L), overweight (96.55±2.90 nmol/L) and obesity (100.18±4.74nmol/L) when compared it with control of normal weight(91.48±2.99), overweight (96.26±5.46 nmol/L) and obesity (102.07±3.66 nmol/L) groups respectively.

There are no significant differences in T4 value among patients of T2DM in different BMI groups, Table (4-12)

As shown in table (4-12), TSH value no different significantly (P <0.05) in patients of normal weight (2.16±0.42), overweight (1.64±0.09 μIU/mL) and obesity (1.4378±0.10 μIU/mL) when compared with control of the normal weight (1.93±0.46 μIU/mL), overweight (1.98±0.24 μIU/mL) and obesity (1.5000±0.18μIU/mL) groups respectively.

The value of TSH among patients of T2DM which increased significantly (P<0.05) in normal weight group comparison to obesity group, While didn’t significant difference between patients of normal weight and overweight

groups, also no significant different between patients of overweight and obesity (Table, 4-12).

Table (4-12): The values of thyroid hormones in control and patients with T2DM(according to BMI) (mean± S.E)

Groups	Parameters	Control	Patients	P-value
Normal weight (18.5-24.9)	T3 nmol/L	(n=8) 1.95±0.05	(n=10) 1.91±0.11 [a]	0.78
	T4 nmol/L	91.48±2.99	101.53±5.59 [a]	0.13
	TSH µIU/mL	1.93±0.46	2.16±0.42[a]	0.71
Overweight (25-29.9)	T3 nmol/L	(n=16) 1.82±0.03	(n=36) 1.77±0.06 [a]	0.49
	T4 nmol/L	96.26±5.46	96.55±2.90 [a]	0.96
	TSH µIU/mL	1.98±0.24	1.64±0.09 [ab]	0.21
Obesity (BMI>30)	T3 nmol/L	(n=20) 1.91±0.08	(n=20) 1.71±.07 [a]	0.08
	T4 nmol/L	102.07±3.66	100.18±4.74 [a]	0.75
	TSH µIU/mL	1.5000±0.18	1.4378±0.10[b]	0.77
LSD	(TSH=0.52)			

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.5.5. Parathyroid Hormone

The results showed PTH value of patients in normal weight (4.09±0.27 pg/mL) decrease significantly ($p<0.05$) in comparison with control (6.19±0.25 pg/mL), while no significant differences ($p<0.05$) in the PTH level in patients of overweight (4.97±0.28 pg/mL) and obesity (6.10±0.67 pg/mL) comparison to control of overweight (4.75±0.40 pg/mL) and obesity (4.68±0.26pg/mL) (Table, 4-13).

As shown in table (4-13) the value of PTH among patients of T2DM which increase significantly in obesity compared to the normal weight group,while no significant differences ($p<0.05$) in the PTH values between patients of

overweight and obesity groups, also no significant differences ($p<0.05$) in the PTH values between patients of normal weight and overweight.

Table (4-13): The value PTH in control and patients with T2DM (according to BMI) (mean± S.E)

Groups	Parameter	Control	Patients	P-value
Normal weight (18.5-24.9)	PTH pg/mL	(n=8)	(n=10)	0.00
		6.19±0.25	4.09±0.27^{*b}	
Overweight (25-29.9)	PTH pg/mL	(n=16)	(n=36)	0.66
		4.75±0.40	4.97±0.28^{ab}	
Obesity (BMI>30)	PTH pg/mL	(n=20)	(n=20)	0.06
		4.68±0.26	6.10±0.67^{a}	
LSD	(PTH=2.01)			

*significant between control and patients at the ($p<0.05$)

Different letters refer to significant differences among patients groups

Similar letters refer to non- significant differences among patients groups

4.6. Correlations Between Level of Osteocacin and Others Parameters Studies in Patients with T2DM:

Correlate bivariate analysis was applied to evaluate the relationship between the levels of OC, insulin and other hormones of patients with T2DM. The results indicate the OC, have a positive correlation with insulin (0.029), total Calcium (0.221), FBG (0.045) and HbA1c (0.005), while it has negative correlation with each of TT (-0.196), LH (-0.171), FSH (-0.128), PRL (-0.137). In addition the OC has inverse correlation with T3, T4, TSH and PTH (-0.112, -0.146, 0.22 and -0.530) respectively (Table, 4-14).

The results indicate that insulin have negative correlation with TT (-0.117) and PRL (-0.190). In addition inverse correlation with T3, T4 and TSH (-0.165,

-0.112 and -0.144) respectively. As well as insulin have also negative correlation with Total Calcium (-0.217) and HbA1c (-0.151). As showed in Table (4-14) insulin has positive correlation with each of LH, FSH, PTH and FBG (0.026), (0.053) and (0.070) respectively.

Table (4-14) showed that TT have a significant (P<0.01) positive correlation with LH (0.368) and significant (P<0.05) positive correlation with PRL (0.260), also have positive correlation with FSH (0.113), T3 (0.189), TSH (0.187), while it has negative correlation with T4, PTH, total Calcium, FBG and HbA1c (-0.021, -.0.024, -0.064, -0.216 and -0.033) respectively.

As shown in Table (4-14) LH have significant (P<0.01) positive correlation with FSH (0.488), also its have positive correlation with PRL (0.027), T3 (0.093), TSH (0.025) and PTH (0.224). As well as the LH have inverse correlation with T4 (-0.005), Calcium (-0.017), FBG (-0.141) and HbA1c (-0.140).

The FSH have positive correlation with each of PRL (0.084), T3 (0.046), TSH (0.068), PTH (0.147), total Calcium (0.020) and HbA1c (0.054) ,and it's have negative correlation with T4 (-0.083) and FBG (-0.011).

As shown in table (4-14) PRL have inverse a significant (P<0.01) correlation with FBG (-0.413), as well as it inverse correlation with T3 (-0.082), T4 (-0.093), and HbA1c (-0.221). As well as PRL have positive correlation with PTH, TSH and Calcium (0.103, 0.101 and 0.024) respectively.

Table (4-14) showed T3 have positive significant (P<0.05) correlation with TSH (0.254) also positive correlation with T4 (0.141), total Calcium (0.115) and FBG (0.070). Furthermore, T3 have negative correlation with both PTH (-0.129) and HbA1c (-0.154).

As shown in table (4-14), T4 have positive correlation with, total Calcium, FBG, and HbA1c (0.030), (0.027) and 0.010) respectively. In addition T4 have inverse correlation with both TSH (-0.072) and PTH (-0.103).

TSH have positive correlation with PTH, total Calcium, FBG and HbA1c (0.031, 0.057, 0.086 and 0.129) respectively.

As shown in table (4-14), PTH have positive correlation with HbA1c (0.034) and inverse correlation with total Calcium (-0.203) and FBG (-0.131). total Calcium have a significant ($P<0.05$) positive correlation with FBG (0.280) and positive correlation with HbA1c (0.089) (Table, 4-14).

FBG as shown in table (4-14) have significant ($P<0.01$) positive correlation with HbA1c (0.644).

Table (4-14) Correlations Between level of osteocalcin and others parameters studies in patients with T2DM

	in	TES	LH	FSH	PRO	T3	T4	TSH	PTH	Ca	FBG	HbA1c
OC	.029	-.196	-.171	-.128	-.137	-.112	-.146	-.022	-.053	.221	.045	.005
In		-.117	.026	.053	-.190	-.165	-.112	-.144	.010	-.217	.070	-.151
TES			.368**	.113	.260*	.189	-.021	.187	-.024	-.064	-.216	-.033
LH				.488**	.027	.093	-.005	.025	.224	-.017	-.141	-.140
FSH					.084	.046	-.083	.068	.147	.020	-.011	.054
PRL						-.082	-.093	.101	.103	.024	-.413**	-.221
T3							.141	.254*	-.129	.115	.070	-.154
T4								-.072	-.103	.030	.027	.010
TSH									.031	.057	.086	.129
PTH										-.203	-.131	.034
Ca											.280*	.089
FBG												.644**

Red Figures are Significant * 0.05, ** 0.01

Chapter Five Discussion

5. Discussion

5.1. Biochemical and Hormonal Parameters between Diabetic Mellitus Patients and Control Groups

The present study showed that the levels of FBG and HbA1c in patients with T2DM which increased significantly ($P<0.05$) in compare with control groups. The elevation in FBG level may be resulting from defects in insulin secretion, insulin action or both (ADA, 2014). The increasing in the level of FBG in diabetic patients was in agreement with many researchers (Abd Ali and Al-Zaidi, 2011; Onah *et al*., 2013; Al-Kaaby and AL-Ali,2020).

The rise in the level of HbA1c was associated with the increasing level of FBG in diabetic groups (Tayde *et al*., 2013).The results showed significant increase in insulin level of T2DM patients in comparison with control group, these findings agree with Maedler *et al*., (2009); Al-Shawk., (2010).

Insulin level is high or normal in the body but the available insulin is insufficient because of insulin resistance (Verma *et al*., 2006). The results show increase OC in patients with T2DM, these results may be due to increase bone resoption process because of insulin resistance which may be lead to increase osteoclast action and OC release to blood or by diabetic pharmacotherapy that make the body cell more responsive to insulin and bone remodeling normal.

The results show decrease PRL in patients with T2DM, these results may be due to diabetic pharmacotherapy which reduced PRL serum level in patients with T2DM(Al-Nami *et al.*,2019). PRL is involved in the regulation of glucose metabolism through regulation the function of pancreatic β-cell and reduces of glucose threshold for insulin secretion (Al-Maiahy *et al.*, 2019).

5.2. Biochemical and Hormonal Parameters (According to Age)

5.2.1. Fasting Blood Glucose (FBG) Level and Glycosylated Hemoglobin A1c (HbA1c)

The results of present study indicated the valuse of FBG and HbA1c in patients with T2DM increased significantly as compare with control groups. The patients with T2DM has high serum glucose, either due to the insulin produced by body did not enough, or because body cells did not respond to the insulin produced (Onahn *et al.*, 2013; ADA, 2014).

The increasing in the level of FBG was in agreement with many researchers Njostad *et al*., (2003), Hussein and Al-Qaisi, (2012) which defined diabetes is a group of metabolic diseases characterized by high level serum glucose. FBG test is directly proportional to the severity of the diabetes mellitus (Ngugi *et al*., 2012).

Glycalted hemoglobin (HbA1c) represents the blood glucose average level within the past 3 months, therefore, increase FBG lead to increase HbA1c in T2DM (Moinuddin and Awanti, 2016).

5.2.2. Calcium

The results of current study showed that Calcium values increased significantly ($P<0.05$) in patients with T2DM of second age group compared to control, these results may be due to increase action of PTH or increase vitamin D that cause increasing calcium absorption from digestive tract. Pittas *et al.*, (2007) reported that Calcium concentration increase with increase vitamin D and action of PTH.

Abbas *et al*., (2012) in their study of 30 patients suffering from T2DM with age (30-70) years and 20 healthy controls in Baghdad province, reported increase Calcium concentrations in patients with T2DM compared to control.

Other age groups did not differ significantly as compared to control, these results agree with study by Abubaker and Mohammed, (2017) which found no significant different in Calcium concentration when compared patients of T2DM with control.

5.2.3. Osteocalcin

The results of this study showed the OC level which increased significantly ($P<0.05$) in patients with T2DM in both second and third age groups compare to the control groups, current study suggest the insulin resistance may be lead to increase bone absoption and increase release OC which storge in extracellular matrix of bone to blood stream, present results disagree with study in Al- Najaf province done by Al-Dujaili and Al-Dujaili, (2016) on T2DM patients (male and women)with age (40-69) years which found decreased OC in T2DM patients. Also the results showed OC level of patients in second and third group increased significantly compare with patients of first group, these results agree with study by Ingram *et al.*, (1994) that reported OC increased with age, while disagreement with study by Alfadda *et al*., (2013) on male have T2DM with age ≥ 40 years that reported OC levels are drop in T2DM subjects.

The results showed no significant different in OC concentration between patients and control in first age group, these results agree with Hwang *et al*., (2012) which reported in their study that T2DM patients (25-60 years) no significant association between T2DM patients and OC.

5.2.4. Insulin

The results of current study show the insulin level in patients of T2DM increased significantly in first age group as comparision with control, these results agree with study done by Yamaguchi and Sugimoto, (2011) that mention patients with T2DM have hyperinsulinemia, due to weakened cellular sensitivity to insulin, and hyperglycemia, due to insulin resistance. So that present study suggests these results due to insulin resistance, while didn't reach to the significant level in patients of second and third groups, these results may be due to use diabetic pharmacotherapy for long time which make changes in insulin levels, by reducing the amount of glucose releases from liver into the blood stream and also make the body cell more responsive to insulin (Inzucchi *et al*., 2012).

The insulin in most people of diabetic patients who are unable to use its glucose for energy because of defects in the insulin action or problem with the cell body's insulin receptors this condition is called insulin resistance (Vital *et al*., 2006; Kim *et al*., 2010).

5.2.5. Reproductive Hormones

The current study results showed that the level of LH in patients with T2DM in third age group increased significantly comparision with control group, these results agrees with Natah *et al*., (2013) that found LH level increase significantly in patients of T2DM because that leydig cells and become resistant to gonadotropin hormone as aresult higher levels of LH.

The results also showed that LH level in the third age group of T2DM which increased significantly in comparision with first age group of T2DM,

these results agree with study by Fukui *et al*., (2007) on male with T2DM (40-69 years) found LH increase with age.

The results showed no significant different in FSH in all groups, these results agree with Achemlal *et al.*, (2005) that found no significant difference in FSH level in T2DM.

The results showed TT level of patients in first, second and third age groups did not different significantly compare with control groups, this results agree with study done by Natah *et al.,* (2013) which found the TT concentration in T2DM did not differ significantly when compare it with control, also agreement with the study done by Esmaeel, (2013) which reported non-significant different in TT levels between T2DM male aged (25-53 yearss) and healthy male in the same age in Babylon province. But TT level of patients with T2DM in third age group increase significantly compared to patients with T2DM in first age group, these results may be related to a hypothalamic defect and/or to an absence of pituitary response to GnRH. Dhindsa *et al*., (2004) demonstrated that 33% (not all) of men with T2DM had significantly lower levels of TT.

PRL concentration decrease significantly in patients with T2DM in first group this results agree with study done by Balbach *et al.,* (2013) that reported decrease PRL level in T2DM, these results may be due to glucose lower drug which may be which inhibits PRL secretion (Hussien *et al.*, 2018).

5.2.6. Thyroid hormones

The results of the present study showed T3 and T4 concentration no significant differences in patients of all age groups compare with the control

groups, these results agrees with study done by Kouidhi *et al.*, (2013) who found that there was no effect of T2DM on thyroid hormones levels.

Hage *et al*., (2011) which reported that reduce in T3 levels has been noted in uncontrolled diabetic patients, this "low T3 state" could be described by impairment in peripheral conversion of T4 to T3 that normalizes with enhancement in glycemic control.

Higher levels of circulating insulin linked with the insulin resistance have shown a proliferative influence on thyroid tissue resulting in larger thyroid size with increased formation of nodules (Ayturk *et al*., 2009).

Results of present study showed the value of TSH no significant difference between patients and control groups, but when we compare among patients; the third group increased in comparison with second group, this results may be because of TSH increasing with aging and it agreement with Boucai and Surks, (2009); Bremner *et al*., (2012).

5.2.7. Parathyroid Hormone

Results of current study showed that PTH level in first age group decreased significantly in comparison to control group, these results may be because of insulin resistance causing inhibitor action on the synthesis and secretion of PTH agree with Martínez *et al*., (1998) which noted that there are inverse correlation between blood glucose and PTH and hyperglycaemia may have an inhibitory action on the synthesis and secretion of PTH, or because decrease in magnesium concentration due to increase urination, agree with Paula *et al*., (2001) that found PTH level decreased in patients with T2DM due to decrease Magnesium concentration which interacts in a complex

manner with PTH, interfering both with the secretion and action of this hormone.

5.3. Biochemical and Hormonal Parameters (According to BMI)

5.3.1. Fasting Blood Glucose and Glycosylated Hemoglobin A1c

The data showed that obesity is common in the subject of T2DM, the results of the present study showed that the diabetic patients have significantly high concentration of FBG and HbA1c than control in all BMI groups and it agree with information that maletioned in ADA, (2015) that diabetic patients FBG level ≥ 126 mg/dl. Increasing of HbA1c levels in our study indicates poor control of FBG levels or poor glycemic index and it agreement with Tayde *et al*., (2013).

Blood glucose is controlled by two main processes: insulin secretion by β-cells in response to a nutrient challenge and insulin action on major target organs, i.e., skeletal muscle, liver and adipose tissue (Ismail, 2010).

Obesity is risk factor for T2DM and the association between obesity and diabetes shown in many studies (Daousi *et al*., 2006; Kumar *et al*., 2008; Tirupathi *et al*., 2015).

5.3.2. Calcium

The results of current study showed Calcium level no different significantly in normal weight, obesity, these results comes in agreement with study done by Nagasaka *et al*., (1995) that found serum calcium concentrations remained unchanged in patients with T2DM, while increase significantly but within the normal range in overweight diabetic group ,these results agree with study done by Al-Selevany (2005)which reported in their

study in Mosul province on 60 T2DM patients with a mean age±SD (47.6±11.6) years and 60 healthy controls with a mean age±SD (35.2±14.3) years that calcium concentration significantly higher in T2DM patients compared with those in healthy controls due to increased flux of Calcium into the extracellular fluid from the skeleton, intestine, and kidney.

The results of present stydy disagree with study in Diwaniya provenice done by Al-Yassin, (2009) on T2DM patients (women and male) with mean age ±SD (56.23 ± 8.25) years and healthy controls of both sexes with a mean age ± SD (36.2+ 14.3) which mentioned that calcium concentration decrease significantly compare to control

5.3.3. Osteocalcin

The results of current study indicated that the OC concentration increased significantly in overweight, and obesity groups, these results agrees with Turkish study done by Küçükler *et al*., (2013) on men with T2DM which found increase OC concentration in T2DM patients, these results may due to insulin has an anabolic effect on osteoblasts (Kream, *et al*., 1985), so our study suggests insulin resistance causes decreasing in bone formation, so increasing bone resorption causing release OC storage in bone to blood or because of diabetic pharmacotherapy that make the body cell more responsive to insulin and bone remodeling are normal

While Lee *et al*., (2007) found no association between BMI and OC level. Results of present study disagreed with Kindblom *et al*., (2009) revealed that OC level was negative correlated with BMI.

5.3.4. Insulin

The current study showed the increasing insulin level significantly in patients with T2DM which suffer from obesity, these results agrees with Kahn and Flier, (2000) which reported obesity may result in hyperinsulinism and insulin resistance, with a close relationship between the increased visceral fat and the degree of insulin resistance as well as hyperinsulinism.

Cubbon *et al.*, (2007) reported that T2DM is most commonly associated with obesity in middle-aged individuals; it is because of defects in insulin receptors on the plasma membrane of cells in target tissues, or an abnormal binding of insulin to receptors.

In the present study there was significant increasing serum insulin in T2DM obese group in comparison to the control group, this is in agrees with. Also, Goran *et al*., (2003) reported that occurrence of obesity was higher in adolescence leading to development of insulin resistance and the duration of obesity was independent risk factor for the development of T2DM. Furthermore, Qatanani and Lazar (2007) reported that obesity main causes of develop insulin resistance in individuals having higher levels of insulin, Finally, Margoni *et al*., (2011) suggested that insulin sensitivity in obese has an important role in the advance of pathogenesis of obesity related insulin resistance.

5.3.5. Reproductive Hormones

Our study showed that there are no significant different in TT, LH and FSH concentrations exception the patients with overweight increase LH concentration significantly in comparison with overweight control, this results is agrees with Dhindsa *et al*., (2018) which reported that One- third of male

with obesity and T2DM have subnormal TT concentrations and gonadotropin concentrations are inappropriately normal. .

The leydig cell responsiveness to exogenous gonadotropin stimulation was attenuated in male with obesity and insulin resistance (Pitteloud *et al*., 2005).

PRL level in patients of normal weight decrease significantly compare with control group and other patients group, this results agrees with study done by Ling *et al*., (2003); Hogan and Stephens, (2005) which found that the human adipose tissue expression PRL mRNA so increase PRL secretion in adipose tissue, also Brandebourg *et al*., (2007) reported PRL increase with BMI. Also gree with Roelfsema *et al*., (2012) which found PRL increase in men with age

5.3.6. Thyroid Hormones

The results of present study showed that T3and T4 levels in all groups no difference significantly in comparison to the control groups, while TSH increase in patients of normal weight group when comparing to the patients of other groups, this results agreement with study done by Nannipieri *et al*., (2009) that found TSH receptors expression in adipose tissue is reduced in obesity and increased with weight loss.

Kouidhi *et al.,* (2013) reported the synthesis of TSH and thyroid hormones may be affected by adipose tissue and caloric consumption, also found correlation between thyroid function and fat accumulation seems to exist, Furthermore, TSH concentrations associated with insulin resistance, which proposes a role of the insulin resistance in controlling thyroid function.

5.3.7. Parathyroid Hormone

The value of PTH increased significantly in patients of obesity group compare with patients of other groups, this results agree with Røislien *et al*., (2011) that reported the PTH level increase in T2DM with BMI, these results may be due decrease vitamin D in obesity due toVitamin D is fat soluble and relationship between PTH and vitamin D is negative.

PTH in the patients with T2DM of normal weight decreased significantly in compare with control group, this results agree with many studies; Kawagishi *et al* ., (1991); Schwarz *et al*., (1992); Ishida *et al*., (1993). Nagasaka *et al*., (1995) reported that serum PTH declined from mid-normal to low-normal levels in patients of T2DM, these results may be due to hyperglycaemia may have an inhibitory action on the synthesis and secretion of PTH.

5.4. Correlations between Levels of Osteocalcin and Others Parameters Studies in Patients with T2DM

The results of this study showed that OC have positive correlation with insulin, but did not reach to the significant, these results may be due to insulin resistance, and due to insulin consider anabolic factor has receptor on osteoblast cell of bone (Yang *et al*., 2010), our study suggests the osteoblasts directly affected by insulin resistance causing increase bone resorption leading to OC release into blood.

This study agrees with Starup-Linde *et al*., (2018) that reported no differences in OC or unOC responses glucose challenges resulting in widely different endogenous insulin and incretin responses.

Also results of this study showed negative correlation between OC and reproductive hormone, but did not reach to the significant, these results disagreemalet with study done by Di Nisio *et al.*, (2017) that reported OC-deficient exhibit increased levels of LH (a pituitary hormone that regulates sex steroid synthesis in the testes).

Astudy by Ducy *et al*., (1996) revealed the existence of parallel endocrine pathway acting on the endocrine function of the testes, which involves OC. The OC effect the TT level through stimulating its production by the testes (Our *et al*., 2011).

Results of the current study showed that the positive correlation between OC and Calcium, but did not reach to the significant. Free OC which available for circulation in the blood may explain the increased concentration of Calcium and OC in the serum of the second group in current study agrees with study done by (Jagtap *et al*., 2011) that reported increasing in Calcium level in serum lead to deficiency of Calcium in bone which may lead to lowering the formation of hydroxyapatite crystals of calcium and OC.Thus, in the state of decreased rate of bone mineralization.

Also results of current study showed OC have positive correlation with FBG and HbA1c but did not reach to significant, these results agrees with Hwang *et al*., (2012) that reported no relationship between total OC and the glucose metabolism. In another studies done by Pietschmann *et al*., (1988) and Küçükler *et al.,* (2014) found no significant association between OC and HbA1c levels.

The results disagree with the meta-analysis by Liu *et al*., (2019) which found an inverse association between OC and FBG in people with and without diabetes.

Result of this study showed that there is significant positive relationship between LH and TT, because of the disruption in the hypothalamic-pituitary-gonadal axis and abnormal testicular energy metabolism (Nna *et al*., 2017). The present study is in line with recent reported by Dhinsda *et al*., (2004) which found positive correlation between LH and TT concentration in male with T2DM.

In health subject negative feedback mechanism between LH and TT, (Du Plessis, *et al*., 2010).

The results of the present study showed a significant positive relationship between PRL and TT; this may be due to disruption in the hypothalamic-pituitary-gonadal axis. In health subjects the relationship are negative between prolactin and testosterone (Zeitlin, 2000).

The results showed negative significant correlation between PRL and FBG, these results which agree with the study done by Balbach *et al*., (2013), and Wang *et al*., (2013) which showed an inverse association between serum PRL levels and FBG in patients with T2DM. Experimaletal studies indicated that PRL has effects on food intake, body weight gain, and insulin resistance via inhibiting adiponectin and IL-6 production in adipose tissue which may lead to T2DM (Nilsson *et al*., 2005; Ben-Jonathan *et al*., 2008).

The experimental studies also showed that PRL has effect on growth of pancreatic ß-cells and reduces threshold for glucose-stimulated insulin

secretion (Petryk *et al*., 2000; Kim *et al*., 2010), which indicate that PRL has a protective effect against T2DM.

The results also showed significant correlation between HbA1c and FBG, these results in agreement with study in Babylon province by Fadhe and Yousif (2019) which found positive correction between HbA1c and FBG

The results also showed that a significant positive correlation between FBG and Calcium, this is may be due to increased increased Calcium absorption from digestive tract or may be due impairment bone mineralization due to diabetes, so we suggests this causes increase Calcium in serum of T2DM

In the individuals heath the relationship betweenTSH and T3 is a negative when TSH increase T3 decrease. The results of present study showed significant positive correlation between T3 and TSH, these results may be due to medications they receive which suppress the level of T4, while raise levels of TSH (Carreras-Gonzalez and Perez, 2007), or suppression of TSH to subnormal levels without clinical symptoms of hyperthyroidism and no change in T_4 or T_3 in patients (Robert *et al*., 2006). The results of present study agree with study done by Datchinamoorthi *et al*., (2006) which found that TSH and T3 have positive relationship in T2DM patients.

The DM influences the thyroid hormones in two sites, first at the level of hypothalamus by controlling TSH release and second at the peripheral tissues by converting T4 to T3 (Chen *et al*., 2007; Vikhe *et al*., 2013).

Chapter Six

Conclusions And Recommendations

6.1. Conclusions

The presents study shows that:

1. Increasing levels of HbAlc and FBG level in patients with T2DM
2. This study revealed increase OC level with BMI and age in patient with T2DM.
3. Increasing PTH levels in serum with increase age and BMI in patients' withT2DM.
4. Increasing insulin levels of T2DM patients with increase BMI.
5. Decrease PRL concentration in patients with T2DM

6.2. Recommendation

1. Future studies included subject with more age of sample and other parameters such as vitamin D and insulin resistance.
2. Comparative study for OC between male with T1DM and other with T2DM
3. Future studies estimule of OC level in adolescence with T1DM.
4. Future studies estimule of OC level in women with other metabolic syndrom such as obesity.

References

References

- **Abbas, W. A; Al-Zubaidi, M.A. and Al-Khazraji, S. K. (2012).** Estimation of serum calcium and parathyroid hormone (PTH) levels in diabetic patients in correlation with age and duration of disease. *Iraqi Journal of Community Medicine*, 25(2): 161-164.
- **Abd Ali, ZA. and Al-Zaidi, MS. (2011).** The Association between Body Mass Index, Lipid Profile and Serum Estradiol Levels in a Sample of Iraqi Diabetic Premenopausal Women. *Oman medical journal*. 4 (26): 263-266.
- **Abu, E. O.; Bord, S.; Horner, A.; Chatterjee, V. K. and Compston, J. E. (1997).** The expression of thyroid hormone receptors in human bone. *Bone*, 21(2): 137-142.
- **Abubaker, N.E. and Mohammed, N. M. (2017)**. serum total calcium, magnesium, potassium and ph in sudanese patients with type 2 diabetes mellitus. *International Journal Of Advanced Research*, 5(3): 86-91
- **Achemlal, L.; Tellal, S.; Rkiouak, F.; Nouijai, A.; Bezza, A.; Derouiche, E. M.; Derouiche, E.; Ghafir, D. and El Maghraoui, A. (2005).** Bone metabolism in male patients with type 2 diabetes. *Clinicalciuml rheumatology*, 24(5): 493-496.
- **Al Rifai, O.; Chow, J.; Lacombe, J.; Julien, C.; Faubert, D.; Susan-Resiga, D.; Essalmani , R.; Creemers, J.; Seidah, N. G.and Ferron, M. (2017).** Proprotein convertase furin regulates osteocalcin and bone endocrine function. *The Journal of clinical investigation*, 127(11): 4104-4117.
- **Al-Aaraji, S. F. (2016).** Serum High-Sensitivity C-Reactive Protein and Endogenous Sex Hormones in Diabetic Male. *Journal of Biotechnology Research Center*, 10(1): 48-52.

- **Al-Dujaili, A. N. G.and Al-Dujaili, H. H. S. (2016)**. Assessment of Osteocalcin Level in Association with Type 2 Diabetic Patients. *Research journal of pharmaceutical biological and chemical sciences,* 7(6):1106-1114.
- **Alfadda, A. A.; Masood, A.; Shaik, S. A.; Dekhil, H.and Goran, M. (2013).** Association between osteocalcin, metabolic syndrome, and cardiovascular risk factors: role of total and undercarboxylated osteocalcin in patients with type 2 diabetes. *International journal of endocrinology*, 2013:1-6
- **Alfadhli, E. M. (2015).** Gestational diabetes mellitus. *Saudi medical journal*, 36(4): 399-406
- **Al-Kaaby, H. H. C.and AL-Ali, Z. A. (2020).** Study of the Effect of Type 2 Diabetes Mellitus on Fertility in Some patients in Misan Province. *Diyala Journal for Pure Science*, *16*(01), 15-28.
- **Allain, T. J.and McGregor, A. M. (1993).** Thyroid hormones and bone. *Journal of Endocrinology*, 139(1): 9-18.
- **Al-Maiahy TJ, Al-Gareeb AI, Al-kuraishy HM (2019)**. Prolactin and risk of preeclampsia: A single institution, cross-sectional study. *Asian Pacific Journal of Reproduction*; 8:112.
- **Al-Nami, M. S.; Al-Kuraishy, H. M., Al-Gareeb, A. I., and Al-Mamoori, F. (2019)**. Metabolic profile and prolactin serum levels in men with type 2 diabetes mellitus: Old-new rubric. *International Journal of Critical Illness and Injury Science*, *9*(3), 120.
- **Al-Selevany, B. K. (2005).** Serum calcium level in patients with type II diabetes mellitus. *Iraqi Journal of Medical Sciences*, *4*(1), 84-88.
- **Al-Shawk, R. (2010).** Study of Some Cytokines Profile in a Sample of Iraqi Type 2 Diabetic Patients and Their Relation with Obesity. PhD. Thesis. IOSR-JDMS. 7: 277-279.

- **AL-Yassin, D. A. M. H. (2009).** Calcium and diabetes mellitus type Two a prospective study done on people with type 2 diabetes in Diwaniya teaching hospital. *KUFA MEDICAL JOURNAL*, *12*(1), 468-475.
- **Amed, S. and Oram, R. (2016)**. Maturity-onset diabetes of the young (MODY): making the right diagnosis to optimize treatment. *Canadian journal of diabetes*, 40(5): 449-454.
- **American Diabetes Association (ADA) (2001).** Hyperglycemic crises in patients with diabetes mellitus 1988–1996. *Diabetes Care* 24:1988–1996.
- **American Diabetes Association (ADA) (2005).** Defining and reporting hypoglycemia in diabetes: a report from the American Diabetes Association Workgroup on Hypoglycemia. *Diabetes Care* 28:1245–1249,
- **American Diabetes Association (ADA) (2010)**. Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 33(Supplement 1): S62-S69.
- **American Diabetes Association (ADA) (2013).** Diagnosis and classification of diabetes mellitus. *Diabetes care*, *36*(Supplement 1), S67-S74.
- **American Diabetes Association (ADA) (2014).** Diagnosis and classification of diabetes mellitus. *Diabetes Care*, 37(Suppl. 1):S81-90.
- **American Diabetes Association (ADA) (2015)**. Classificaltion and Diagnosis of Diabetes. *Diabetes Care*, 38 (Suppl. 1):S8–S16.
- **American Diabetes Association (ADA) (2016).** 2. Classification and diagnosis of diabetes. *Diabetes care*, *39*(Supplement 1), S13-S22.
- **American Diabetes Association (ADA) (2018).** 2. Classification and diagnosis of diabetes: standards of medical care in diabetes—2018. *Diabetes care*, *41*(Supplement 1), S13-S27.

- **American Diabetes Association (ADA) (2019).** Classification and Diagnosis of Diabetes: Standards of Medical Care in Diabetes. *Diabetes Care*, 42(Suppl. 1):S13–S28.
- **Ardawi, M. S. M.; Akhbar, D. H.; AlShaikh, A.; Ahmed, M. M.; Qari, M. H.; Rouzi, A. A.; Ali, A. Y.; Abdulrafee,A.A. and Saeda, M. Y. (2013).** Increased serum sclerostin and decreased serum IGF-1 are associated with vertebral fractures among postmenopausal women with type-2 diabetes. *Bone*, 56(2): 355-362.
- **Ardawi, M. S. M.; Maimani, A. A.; Bahksh, T. A.; Rouzi, A. A.; Qari, M. H. and Raddadi, R. M. (2010).** Reference intervals of biochemical bone turnover markers for Saudi Arabian women: a cross-sectional study. *Bone*, 47(4): 804-814.
- **Asokan, A. G.; Jayakumar Jaganathan, R. P.; Soman, R. R.; Sebastian, S. T.and Pullishery, F. (2017)**. Evaluation of bone mineral density among type 2 diabetes mellitus patients in South Karnataka. *Journal of natural science, biology, and medicine*, 8(1): 94-98.
- **Atkinson, M.and Maciarnen, N. (1990):** ″What causes diabetes?″ *Scientific American*, 263(1): 62-71
- **Ayturk, S.; Gursoy, A.; Kut, A.; Anil, C.; Nar, A. and Tutuncu, N. B. (2009).** Metabolic syndrome and its components are associated with increased thyroid volume and nodule prevalence in a mild-to-moderate iodine-deficient area. *European Journal of Endocrinology*, 161(4): 599-605.
- **Balbach, L.; Wallaschofski, H.; Völzke, H.; Nauck, M.; Dörr, M. and Haring, R. (2013).** Serum prolactin concentrations as risk factor of metabolic syndrome or type 2 diabetes?. *BMC endocrine disorders*, 13(1):1-8

- **Barbour, L. A.; McCurdy, C. E.; Hernandez, T. L.; Kirwan, J. P.; Catalano, P. M.and Friedman, J. E. (2007).** Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes care*, 30(Supplement 2): S112-S119.
- **Basu, S.; Yudkin, J. S.; Kehlenbrink, S.; Davies, J. I.; Wild, S. H.; Lipska, K. J.; Sussman ,J. B .and Beran, D. (2019)**. Estimation of global insulin use for type 2 diabetes, 2018–30: a microsimulation analysis. *The Lancet Diabetes and Endocrinology*, 7(1): 25-33.
- **Ben-Dov, I.Z.; Galitzer ,H.; Lavi-Moshayoff, V.; Goetz ,R.; Kuro-o, M.; Mohammadi ,M.; Sirkis ,R.; Naveh-Many, T. and Silver ,J. (2007)**. The parathyroid is a target organ for FGF23 in rats. *Journal of Clinical Investigation*, 117(12):4003–4008.
- **Ben-Jonathan, N.; LaPensee, C. R.and LaPensee, E. W. (2007).** What can we learn from rodents about prolactin in humans?. *Endocrine reviews*, 29(1): 1-41.
- **Bilotta, F. L.; Arcidiacono, B.; Messineo ,S.; Greco, M.; Chiefari, E.; Britti, D.; Nakanishi, T.; Foti, D. P.and Brunetti, A. (2018).** Insulin and osteocalcin: further evidence for a mutual cross-talk. *Endocrine*, 59(3): 622–632.
- **Bluestone, J. A.; Herold, K.and Eisenbarth, G. (2010).** Genetics, pathogenesis and clinical interventions in type 1 diabetes. *Nature*, 464(7293):1293-1300.
- **Bonewald, L. F. (2011).** The amazing osteocyte. *Journal of bone and mineral research*, 26(2): 229-238.
- **Bonneau, J.; Ferland, G.; Karelis, A. D.; Doucet, E.; Faraj, M.; Rabasa-Lhoret, R.and Ferron, M. (2017)**. Association between osteocalcin gamma-

carboxylation and insulin resistance in overweight and obese postmenopausal women. *Journal of Diabetes and its Complications*, 31(6): 1027-1034.

- **Boucai, L.and Surks, M. I. (2009).** Reference limits of serum TSH and free T4 are significantly influenced by race and age in an urban outpatient medicalpractice. *Clinical endocrinology*, 70(5): 788-793.
- **Brandebourg, T.; Hugo, E. and Ben-Jonathan, N. (2007).** Adipocyte prolactin: regulation of release and putative functions. *Diabetes, Obesity and Metabolism*, 9(4): 464-476.
- **Bremner, A. P.; Feddema, P.; Leedman, P. J.; Brown, S. J.; Beilby, J. P.; Lim, E. M.; Wilson ,S.G.; O'Leary ,P. C.and Walsh, J. P. (2012).** Age-related changes in thyroid function: a longitudinal study of a community-based cohort. *The Journal of Clinical Endocrinology*, 97(5): 1554-1562.
- **Brownstein, C.A.; Adler, F.; Nelson-Williams, C.; Iijima, J.; Li, P.; Imura, A.; Nabeshima, Y.; Reyes-Mugica, M.; Carpenter,T.O. and Lifton, R.P. (2008).** A translocation causing increased alpha-klotho level results in hypophosphatemic rickets and hyperparathyroidism. *Proceedings of the National Academy of Sciences*, 105(9): 3455-3460.
- **Burns, K. H.and Matzuk, M. M. (2002)**. Minireview: genetic models for the study of gonadotropin actions. *Endocrinology*, 143(8): 2823-2835.
- **Carnevale, V.; Romagnoli, E.; D'Erasmo, L.and D'Erasmo, E. (2014).** Bone damage in type 2 diabetes mellitus. *Nutrition, Metabolism and Cardiovascular Diseases*, 24(11): 1151-1157.
- **Carreras-González, G.and Pérez, A. (2007).** Thyroid Autoimmunity at Onset of Type 1 Diabetes as a Predictor of Thyroid Dysfunction: Response to Warren and Frier. *Diabetes Care*, *30*(11), e121-e121.

- **Černe, D. (2002).** Early Detection of Chronic Complications: Diabetic Nephropathy. *EJIFCC*, *13*(5), 194-199.
- **Chandrashekar, V. (2016)**. HbA1c Separation by High Performance Liquid Chromatography in Hemoglobinopathies. *Scientifical*, 2016:1-4
- **Chang, Y. H.; Lei, C. C.; Lin, K. C.; Chang, D. M.; Hsieh, C. H. and Lee, Y. J. (2016).** Serum uric acid level as an indicator for CKD regression and progression in patients with type 2 diabetes mellitus—a 4.6-year cohort study. *Diabetes/metabolism research and reviews*, *32*(6), 557-564.
- **Chen, H. S.; Wu, T. E.; Jap, T. S.; Lu, R. A.; Wang, M. L.; Chen,R. L.and Lin, H. D. (2007).** Subclinical hypothyroidism is a risk factor for nephropathy and cardiovascular diseases in Type 2 diabetic patients. *Diabetic Medicine*, 24(12): 1336-1344.
- **Cho, N.; Shaw, J. E.; Karuranga, S.; Huang, Y.; da Rocha Fernandes, J. D.; Ohlrogge, A. W. and Malanda, B. (2018).** IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes research and clinical practice*, 138: 271-281.
- **Clemens, T. L.and Karsenty, G. (2011).** The osteoblast: an insulin target cell controlling glucose homeostasis. *Journal of Bone and Mineral Research*, 26(4): 677-680.
- **Cryer, P. E. (2006).** Hypoglycemia in diabetes: pathophysiological mechanisms and diurnal variation. *Progress in brain research*, *153*, 361-365.
- **Cubbon, R. M.; Rajwani, A. and Wheatcroft, S. B. (2007)**. The impact of insulin resistance on endothelial function, progenitor cells and repair. *Diabetes and Vascular Disease Research*, 4(2): 103-111.
- **Dallas, S. L.; Prideaux, M. and Bonewald, L. F. (2013).** The osteocyte: an endocrine cell… and more. *Endocrine reviews*, 34(5): 658-690.

- **D'Amelio, P.; Sassi, F.; Buondonno, I.; Spertino, E.;Tamone, C.; Piano, S.; Zugna, D. ; Richiardi, L . Isaia, G. C. (2015).** Effect of intermittent PTH treatment on plasma glucose in osteoporosis: a randomized trial. *Bone*, 76: 177-184.
- **Daousi, C.; Casson, I.; Gill, G.; Macfarlane, I.; Wilding, J. and Pinkney, J. (2006).** Prevalence of obesity in type2 diabetes patients in secondary care: association with cardiovascular risk factors. *Postgraduate medical journal*, 82(966): 280-284
- **Datchinamoorthi, S.; Rathanavel, N.; Rajagopalan, B .and Vanaja R.(**2016): Study of Thyroid Dysfunction in Type II Diabetes Mellitus. *International Journal of Pharmaceutical Sciences and Research*, 7(9): 3877-3880
- **Datta, H. K.; Ng, W. F.; Walker, J. A.; Tuck, S. P. and Varanasi, S. S. (2008).** The cell biology of bone metabolism. Journal of clinical pathology, 61(5): 577-587.
- **De Toni, L.; Guidolin, D.; De Filippis, V.; Tescari, S.; Strapazzon, G.; Santa, R.M.; Ferlin, A.; Plebani, M. and Foresta, C. (2016).** Osteocalcin and sex hormone binding globulin compete on a specific binding site of GPRC6A. *Endocrinology*, 157(11): 4473-4486.
- **Dhaliwal, R.; Cibula, D.; Ghosh, C.; Weinstock, R. S.and Moses, A. M. (2014).** Bone quality assessmalet in type 2 diabetes mellitus. *Osteoporosis international*, 25(7): 1969-1973.
- **Dhindsa, S.; Ghanim, H.; Batra, M.and Dandona, P. (2018).** Hypogonadotropic hypogonadism in male with diabesity. *Diabetes Care*, 41(7): 1516-1525.

- **Dhindsa, S.; Prabhakar, S.; Sethi, M.; Bandyopadhyay, A.; Chaudhuri, A., and Dandona, P. (2004).**Frequent occurrence of hypogonadotropic hypogonadism in type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, 89(11): 5462-5468.
- **Di Nisio, A.; Rocca, M. S.; Fadini, G. P.; De Toni, L.; Marcuzzo, G.; Marescotti, M. C.; Sanna, M.; Plebani, M.; Vettor, R.; Avogaro, A. and Foresta, C. (2017)**. The rs2274911 polymorphism in GPRC 6A gene is associated with insulin resistance in normal weight and obese subjects. *Clinical endocrinology*, 86(2): 185-191.
- **Diamanti-Kandarakis, E.; Livadas, S.;Katsikis, I.; Piperi, C.; Aimilia, M., Papavassiliou, A. G.and Panidis, D. (2011).** Serum concentrations of carboxylated osteocalcin are increased and associated with several components of the polycystic ovarian syndrome. *Journal of bone and mineral metabolism*, 29(2): 201-206.
- **Dobnig, H.; Piswanger-Sölkner, J. C.; Roth, M.; Obermayer-Pietsch, B.; Tiran, A.; Strele, A.; Maier, E.; Maritschnegg ,P.; Sieberer ,C.and Fahrleitner-Pammer, A. (2006)**. Type 2 diabetes mellitus in nursing home patients: effects on bone turnover, bone mass, and fracture risk. *The Journal of Clinical Endocrinology and Metabolism*, 91(9): 3355-3363.
- **Downey, P. A. and Siegel, M. I. (2006).** Bone biology and the clinical implications for osteoporosis. Physical therapy, 86(1), 77-91.
- **Drucker, D. J. (2006)**. The biology of incretin hormones. *Cell metabolism*, 3(3): 153-165.
- **Du Plessis, S. S.; Cabler, S.; Mcalister, D. A.; Sabanegh, E.and Agarwal, A. (2010).** The effect of obesity on sperm disorders and male infertility. *Nature Reviews Urology*, 7(3): 153-161.

- **Ducy, P. (2011).** The role of osteocalcin in the endocrine cross-talk between bone remodelling and energy metabolism. *Diabetologia*,54 (6):1291–1297.
- **Ducy, P.; Desbois, C.; Boyce, B.; Pinero, G.; Story, B.; Dunstan, C.; Smith, E.; Bonadio, J.; Goldstein, S.; Gundberg, C.and Bradley, A. (1996).** Increased bone formation in osteocalcin-deficient mice. *Nature*, 382(6590): 448-452.
- **El, K. B. and Azar, S. T. (2013).**Low testosterone and diabetes. *Current diabetes reviews*, 9(5): 418-421.
- **Endres, D.B and Rude, R.K. (2006).** Mineral and Bone Metabolism. In: Burtis ,C.A.; Ashwood ,E.R. and Bruns ,ED, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th ed. St. Louis (MO): Saunders Elsevier:1891-1965.
- **Esmaeel, A. K. (2013).** Relationships between diabetes mellitus type 2 and male testosterone level. *Journal of Babylon University/Pure and Applied Sciences*, 21(1).
- **Everts, V.; Delaisse, J. M.; Korper, W.; Jansen, D. C.; Tigchelaar-Gutter, W.; Saftig, P.and Beertsen, W. (2002).** The bone lining cell: its role in cleaning Howship's lacunae and initiating bone formation. *Journal of Bone and Mineral Research*, 17(1): 77-90.
- **Fadhe, A. A. and Yousif, A. K. (2019).** Correlation of Glycated Hemoglobin (Hba1c) and Serum Uric Acid in Type-2 Diabetic Patients. *Indian Journal of Public Health Research and Development*, *10*(5), 1250-1254.
- **Fahie-Wilson, M.; Brunsden, P.;Surrey, J.and Everitt, A. (2000).** Macroprolactin and the Roche Elecsys prolactin assay: characteristics of the reaction and detection by precipitation with polyethylene glycol. *Clinical Chemistry*, 46(12): 1993-1995.

- **Faienza, M. F.; Luce, V., Ventura; A., Colaianni, G.; Colucci, S.; Cavallo, L. Grano, M.and Brunetti, G. (2015).** Skeleton and glucose metabolism: a bone-pancreas loop. *International journal of endocrinology*, 2015(758148): 1-5.
- **Fares, J. E.; Choucair, M.; Nabulsi, M.; Salamoun, M.; Shahine, C. H.and Fuleihan, G. E. H. (2003)**. Effect of gender, puberty, and vitamin D status on biochemical markers of bone remodededeling. *Bone*, 33(2): 242-247.
- **Farr, J. N.and Khosla, S. (2016).** Determinants of bone strength and quality in diabetes mellitus in humans. *Bone*, 82(15): 28-34.
- **Fekete, C. and Lechan, R. M. (2013).** Central regulation of hypothalamic-pituitary-thyroid axis under physiological and pathophysiological conditions. *Endocrine reviews*, 35(2): 159-194.
- **Ferron, M. (2018)**. Endocrine Functions of Bone. In: Belfiore, A. and LeRoith, D. (ed) Principles of Endocrinology and Hormone Action, Springer Internationa, switzerand. pp,559-580
- **Ferron, M.; Wei, J.; Yoshizawa, T.; Del Fattore, A.; DePinho, R. A.; Teti, A.; Ducy ,P and Karsenty, G. (2010).** Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell*, 142(2): 296-308.
- **Ferron, M.and Lacombe, J. (2014).** Regulation of energy metabolism by the skeleton: osteocalcin and beyond. Archives of biochemistry and biophysics, 561(2014):137-146.
- **Flaxman, S.R.; Bourne, R. R.; Resnikoff, S.; Ackland, P.; Braithwaite, T.; Cicinelli, M.V. and Leasher, J. (2017).** Global causes of blindness and distance vision impairment 1990–2020: a systematic review and meta-analysis. *The Lancet Global Health*, *5*(12), e1221-e1234.
- **Fletcher, E. L.; Phipps, J. A.; Ward, M. M.; Puthussery, T.and Wilkinson-Berka, J. L. (2007).** Neuronal and glial cell abnormality as

predictors of progression of diabetic retinopathy. *Current pharmaceutical design*, *13*(26), 2699-2712.

- **Fukui, M.; Soh, J.; Tanaka, M.; Kitagawa, Y.; Hasegawa, G., Yoshikawa, T.; Miki,T. and Nakamura, N. (2007).** Low serum testosterone concentration in middle-aged male with type 2 diabetes. *Endocrine journal*, 54 (2007): 1-10.
- **Gandhi, A.; Beam, H. A.; O'Connor, J. P.; Parsons, J. R.and Lin, S. S. (2005).** The effects of local insulin delivery on diabetic fracture healing. *Bone*, 37(4): 482-490.
- **Gerdhem, P.; Isaksson, A.; Åkesson, K.and Obrant, K. J. (2005).** Increased bone density and decreased bone turnover, but no evident alteration of fracture susceptibility in elderly women with diabetes mellitus. *Osteoporosis International*, 16(12): 1506-1512.
- **Glover, S. J.; Garnero, P.; Naylor, K.; Rogers, A.and Eastell, R. (2008).** Establishing a reference range for bone turnover markers in young, healthy women. *Bone*, 42(4): 623-630.
- **Goldenburg, R. and Punthakee, Z. (2013)** . Canadian Diabetes Association Clinical Practice Guidelines for the Prevention and Management of diabetes in Canada. Definition, classification and diagnosis of diabetes, pre-diabetes, and metabolic syndrome. Can. *J. Diabetes.* 37(1): 8-11
- **Goldstein, D. E.; Little, R. R.; Lorenz, R. A.; Malone, J. I.; Nathan, D.; Peterson, C. M. and Sacks, D. B. (2004).** Tests of glycemia in diabetes. *Diabetes care*, *27*(7), 1761-1773.
- **Goran, M. I.; Ball, G. D.and Cruz, M. L. (2003).** Obesity and risk of type 2 diabetes and cardiovascular disease in children and adolescents. *The Journal of Clinical Endocrinology and Metabolism*, 88(4): 1417-1427.

- **Gouveia, C. H.; Schultz, J. J.; Bianco, A. C.and Brent, G. A. (2001).** Thyroid hormone stimulation of osteocalcin gene expression in ROS 17/2.8 cells is mediated by transcriptional and post-transcriptional mechanisms. *Journal of Endocrinology*, 170(3): 667-675.
- **Guitar-Bryan, L. and Bryan, J. (2008).** Neonatal diabetes mellitus. *Endocrine reviews*, 29(3): 265-291.
- **Gundberg, C. M.; Looker, A. C.; Nieman, S. D.and Calvo, M. S. (2002).** Patterns of osteocalcin and bone specific alkaline phosphatase by age, gender, and race or ethnicity. *Bone*, 31(6): 703-708.
- **Haffner, S. M.; Lehto, S.; Rönnemaa, T.; Pyörälä, K.and Laakso, M. (1998).** Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *New England journal of medicine*, *339*(4), 229-234..
- **Hage, M.; Zantout, M. S.and Azar, S. T. (2011).** Thyroid disorders and diabetes mellitus. *Journal of thyroid research*, 2011:1-7
- **Hamann, C.; Kirschner, S.; Günther, K. P.and Hofbauer, L. C. (2012).** Bone, sweet bone—osteoporotic fractures in diabetes mellitus. *Nature Reviews Endocrinology*, 8(5): 297.
- **Harada, S. I. and Rodan, G. A. (2003).** Control of osteoblast function and regulation of bone mass. *Nature*, 423(6937): 349–355.
- **Harsløf, T.; Sikjaer, T.; Sørensen, L.; Pedersen, S. B.; Mosekilde, L.; Langdahl, B. L.and Rejnmark, L. (2015).** The effect of treatmalet with PTH on undercalciumrboxylated osteocalciumlcin and energy metabolism in hypoparathyroidism. *The Journal of Clinicalciuml Endocrinology and Metabolism*, 100(7): 2758-2762.

- **Hauschka, P. V.; Lian, J. B.; Cole, D. E. and Gundberg, C. M. (1989).** Osteocalcin and matrix Gla protein: vitamin K-dependent proteins in bone. *Physiological reviews*, 69(3): 990-1047.
- **Helrich, K. (1990)**. Official Methods of analysis of the Association of Official Analytical Chemisty.
- **Hernández-Gil, I.T.; Gracia, M.A.; Pingarrón, M.D. and Jerez, L.B (2006)** Physiological basis of bone regeneration I. Histology and physiology of bone tissue. *Medicina Oral Patologia Oral y Cirugia Bucal.* 11: E47 -51
- **Hinoi, E.; Gao, N.; Jung, D. Y.; Yadav, V.; Yoshizawa, T.; Myers, M. G.; Chua, J.S.C.; Kim, J.K. and Karsenty, G. (2008).** The sympathetic tone mediates leptin's inhibition of insulin secretion by modulating osteocalcin bioactivity. *Journal Cell Biology*, 183(7): 1235-1242.
- **Hoang, Q. Q.; Sicheri, F.; Howard, A. J. and Yang, D. S. (2003).** Bone recognition mechanism of porcine osteocalcin from crystal structure. *Nature*, *425*(6961), 977-980.
- **Hofbauer, L. C.; Brueck, C. C.; Singh, S. K.and Dobnig, H. (2007).** Osteoporosis in patients with diabetes mellitus. *Journal of Bone and Mineral Research*, 22 (9): 1317-1328.
- **Hofstra, J.; Loves, S.; Van Wageningen, B.; Ruinemans-Koerts, J.; Jansen, I.and De Boer, H. (2008).** High prevalence of hypogonadotropic hypogonadism in male referred for obesity treatment. *Netherland Journal Medicine*, 66(3): 103-109.
- **Hogan, J.C. and Stephens, J.M. (2005).** The Regulation of Fatty Acid Synthase by STAT5A. *Diabetes*, 54(7):1968-1975.
- **Hough, F. S.; Pierroz, D. D.; Cooper, C. and Ferrari, S. L. (2016).** Mechanisms in endocrinology: mechanisms and evaluation of bone fragility in

type 1 diabetes mellitus. *European journal of endocrinology*, 174(4): R127-R138.

- **Hussein, Z., and Al-Qaisi, J. (2012).** Effect of diabetes mellitus type 2 on pituitary gland hormones (FSH, LH) in male and women in Iraq. *Al-Nahrain Journal of Science*, 15(3): 75-79.
- **Hussien, N.R.; Al-Naimi, M.S.; Rasheed, H.A.; Al-Kuraishy ,H.M. and Al-Gareeb, A.(2018).** Sulfonylurea and neuroprotection: The bright side of the moon. *Journal of Advanced Pharmaceutical Technology and Research*. 2018;9:120–3
- **Hwang, Y. C.; Jee, J. H.; Jeong, I. K.; Ahn, K. J.; Chung, H. Y.and Lee, M. K. (2012).** Circulating osteocacin level is not associated with incident type 2 diabetes in middle-aged male subjects: mean 8.4-years retrospective follow-up study. *Diabetes Care*, 35(9): 1919-1924.
- **Im, J. A.; Yu, B. P.; Jeon, J. Y.and Kim, S. H. (2008)**. Relationship between osteocalcin and glucose metabolism in postmenopausal women. *Clinical Chimical Acta*, 396(1-2): 66-69.
- **Ingram, R. T.; Park, Y. K.; Clarke, B. L.and Fitzpatrick, L. A. (1994).** Age-and gender-related changes in the distribution of osteocalcin in the extracellular matrix of normal male and female bone. Possible involvement of osteocalcin in bone remodeling. *The Journal of clinical investigation*, 93(3): 989-997.
- **Inzucchi, S., Bergenstal, R., Buse, J., Diamant, M., Ferrannini, E., Nauck, M., Peters, A., Tsapas, A., Wender, R. and Matthews, D. (2010).** Management of hyperglycemia in type 2 diabetes. ADA. 35(6): 1364-1379.
- **Ishida, H.; Suzuki, K.; Someya, Y.; Nishimura, M.; Sugimoto, C.; Goto, M.; Taguchi, Y.; Kasahara, H.; Kadowaki, S.; Imura, H.and Seino, Y.**

(1993) .Possible role of parathyroid hormone-related peptide on maintenance of calcium homeostasis in patients with non-insulin-dependent diabetes mellitus. *European Journal of Endocrinology*, 129(6): 519-524.

- **Ismail, B. H. (2010).** Comparison Study of insulin level and lipid profile in diabetes mellitus in Ramady city. *Journal of university of Anbar for Pure science*, *4*(2), 13-15.
- **Ivaska, K. K.;Hentunen, T. A.;Vääräniemi, J.; Ylipahkala, H.; Pettersson, K. and Väänänen, H. K. (2004).** Release of intact and fragmented osteocalcin molecules from bone matrix during bone resorption in vitro. *Journal of Biological Chemistry*, 279(18):18361-18369.
- **Jackulia, P. and Payer, J. (2014).** Osteoporosis, fractures, and diabetes. *International Journal of Endocrinology* 2014(3):1-7
- **Jafari, S. M.; Nabipour, I.; Larijani, B.; Amiri, M.and Amiri, Z. (2008).** Reference database of CrossLaps and osteocalcin for a healthy Iranian population. *Archives of Iranian Medicine*, 11(2):203-206.
- **Jagtap, V. R.; Ganu, J. V.and Nagane, N. S. (2011).** BMD and serum intact osteocacin in postmenopausal osteoporosis women. *Indian Journal of Clinical Biochemistry*, 26(1): 70-73.
- **Kahn, B. B.and Flier, J. S. (2000).** Obesity and insulin resistance. *The Journal of clinical investigation*, 106(4): 473-481.
- **Kainuma, S.; Tokuda, H.; Fujita, K.; Kawabata, T.; Sakai, G., Matsushima-Nishiwaki, R.; harada ,A.; kozawa,O. and Otsuka, T. (2016).** Attenuation by incretins of thyroid hormone-stimulated osteocalcin synthesis in osteoblasts. *Biomedical reports*, 5(6): 771-775.
- **Kanazawa, I. (2015).** Osteocalcin as a hormone regulating glucose metabolism. *World journal of diabetes*, 6(18): 1345-1354.

- **Karsenty, G. (2011).** Bone endocrine regulation of energy metabolism and male reproduction. *Comptes rendus biologies*, 334(10): 720-724.
- **Karsenty, G.and Ferron, M. (2012).** The contribution of bone to whole-organism physiology. *Nature*, 481(7381): 314-320.
- **Karsenty, G.and Oury, F. (2014).** Regulation of male fertility by the bone-derived hormone osteocalcin. *Molecular and cellular endocrinology*, 382(1): 521-526.
- **Kawagishi, T.; Morii, H.; Nakatsuka, K.; Sasao, K.; Kawasaki, K.; Miki, T.; Nishizawa, Y. (1991)**. Parathyroid hormone secretion in diabetes mellitus. In: Morii, H (ed) Calcium-Regulating Hormones. I.Role in Disease and Aging. *Basel, Karger, Contributions to nephrology*, 90: 217-222
- **Khosla ,S. and Melton ,L.J.(1995)**. Secondary osteoporosis. In: Osteoporosis; Etiology, Diagnosis and Management. 2nd edition. Riggs ,L.G. and Melton ,L.J. (eds). Lippincott-Raven Publishers, Philadelphia, pp183-204.
- **Kim, H.; Toyofuku, Y.; Lynn, F.C.; Chak, E.; Uchida, T.; Mizukami, H.; Fujitani, Y.; Kawamori, R.; Miyatsuka, T.; Yang, K.; Kosaka, Y.; Honig, G.; Hart, M. V.; Kishimoto, N.; Wang, J.; Yagihashi, S.; Tecott, L.H.; Watada, H.; German, M.S(2010).** Serotonin regulates pancreatic beta cell mass during pregnancy. *Nature medicine*, 16(7): 804808 -
- **Kindblom, J. M.; Ohlsson, C.; Ljunggren, Ö.; Karlsson, M. K.; Tivesten, Å.; Smith, U.and Mellström, D. (2009)**. Plasma osteocalcin is inversely related to fat mass and plasma glucose in elderly Swedish male. *Journal of Bone and Mineral Research*, 24(5): 785-791.
- **Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN (July 2009).** "Hyperglycemic crises in adult patients with diabetes". *Diabetes Care.* 32 (7): 1335-1343.

- **Kitabchi, A. E.; Umpierrez, G. E.; Murphy, M. B.and Kreisberg, R. A. (2006).** Hyperglycemic crises in adult patients with diabetes: a consensus statement from the American Diabetes Association. *Diabetes care*, *29*(12), 2739-2748.
- **Kitabchi, A.E.; Umpierrez, G.E.; Murphy, M. B.; Barrett, E. J.; Kreisberg, R. A.; Malone, J. I. and Wall, B. M. (2001).** Management of hyperglycemic crises in patients with diabetes. *Diabetes care*, *24*(1), 131-153.
- **Kopp, P. (2005).** Thyroid hormone synthesis. The Thyroid: Fundamental and Clinical Text, 9th, Braverman, L.E.; Utiger, R.D. Lippincott Williams and Wilkins, Philadelphia pp.52.
- **Kouidhi, S.; Berhouma, R.; Ammar, M.; Rouissi, K.; Jarboui, S.; Clerget-Froidevaux, M. S.; Seugnet, I.; Abid, H.; Bchir, F.; Demaleeix, B.; Guissouma, H.and Guissouma, H. (2013)**. Relationship of thyroid function with obesity and type 2 diabetes in euthyroid Tunisian subjects. *Endocrine research*, 38(1): 15-23.
- **Kream, B. E.; Smith, M. D.; Canalis, E.and Raisz, L. G. (1985).** Characterization of the effect of insulin on collagen synthesis in fetal rat bone. *Endocrinology*, 116(1): 296-302.
- **Küçükler, F. K.; Bardak, S.; Çelik, T.; Tütüncü, Y.; Akbaba, G.; Işık, S.; Karaahmetoğlu, S.; Eskioğlu, E.; Berker, D.; Güler, S. (2014).** No relationship between osteocalcin and the microvascular complications of type 2 diabetes. *Turkish Journal of Endocrinology and Metabolism*, 18(12): 126-131.
- **Kumar, S.; Mukherjee, S.; Mukhopadhyay, P.; Pandit, k.; Raychaudhuri, M.; Sengupta, N.; Ghosh, H.; Sarkar, S.; Mukherjee, S. and Chowdhury, S. (2008).** Prevalence of diabetes and impaired fast glucose a

selected population special reference to influence of family history and anthropometric measurements The Kolkata police study. *JAPI*. 56: 841-844.

- **Lacombe, J.; Karsenty, G.and Ferron, M. (2013).** In vivo analysis of the contribution of bone resorption to the control of glucose metabolism in mice. *Molecular metabolism*, 2(4): 498-504.
- **Lebovitz, H. E. (2004).** Diagnosis, classification and pathogenesis of diabetes mellitus. *The Journal of clinical psychiatry*. 62(27): 5-9.
- **Lee, N. K.; Sowa, H.; Hinoi, E.; Ferron, M.; Ahn, J. D.; Confavreux, C.; Dacquin, R.; Mee ,P.J.; McKee ,M.D.; Jung, D.Y.and Zhang, Z. (2007).** Endocrine regulation of energy metabolism by the skeleton. *Cell*, 130(3): 456-469.
- **Liao, C. C.; Lin, C. S.; Shih, C. C.; Yeh, C. C.; Chang, Y. C.; Lee, Y. W.and Chen, T. L. (2014)**. Increased risk of fracture and postfracture adverse events in patients with diabetes: two nationwide population-based retrospective cohort studies. *Diabetes Care*, 37(8): 2246-2252.
- **Ling, C.; Svensson, L.; Odén, B.; Weijdegård, B.; Edén, B.; Edén, S. and Billig, H. (2003).** Identification of Functional Prolactin (PRL) Receptor Gene Expression: PRL Inhibits Lipoprotein Lipase Activity in Human White Adipose Tissue. *The Journal of Clinical Endocrinology and Metabolism*, 88(4): 1804-1808.
- **Liu, D. M.; Guo, X. Z.; Tong, H. J.; Tao, B.; Sun, L. H.; Zhao, H. Y.; Ning, G .and Liu, J. M. (2015).** Association between osteocalcin and glucose metabolism: a meta-analysis. Osteoporosis International, 26(12): 2823-2833.
- **Liu, Y.; Liu, X.; Lewis, J. R.; Brock, K.; Brennan-Speranza, T. C.; and Teixeira-Pinto, A. (2019).** Relationship between serum

osteocalcin/undercarboxylated osteocalcin and type 2 diabetes: a systematic review/meta-analysis study protocol. *BMJ open,* 9(3): e023918.

- **Lu, N.; Sun, H.; Yu, J.; Wang, X.; Liu, D.; Zhao, L.; Sun, L.; Zhao, H.; Tao, B. and Liu, J. (2015)**. Glucagon-like peptide-1 receptor agonist Liraglutide has anabolic bone effects in ovariectomized rats without diabetes. *PloS one*, 10(7):1-15.
- Maedler, K., Dharmadhikari, G., Schumann, D. and Størling, J. (2009). Interleukin-1 beta targeted therapy for type 2 diabetes. *Expert opinion on biological therapy*. 9(9): 1177-88.
- **Margoni, A.; Perrea, D.N.; Vlachos, I. and Prokopaki, G. (2011).** Serum Leptin, Adiponectin and Tuomour Necrosis factor in hyperlipidemic rats with/without Concomitant Diabetes Mellitus. *Molecular Medicine*, 17(1-2): 36-40
- **Marshall, SM. (2010).** Standardization of HbA1c. good or bad? Nat. *Rev. Endocrinol* 6. 408-411.
- **Martin ,A.(2006).** Clinical chemistry and metabolic medicine. 7th ed. London: Hodder Arnold: pp 432
- **Martínez ,I.; Saracho, R.; Moina, I.;Montenegro ,J.and Llach, F.(1998)**. Is there a lesser hyperparathyroidism in diabetic patients with chronic renal failure?. *Nephrol Dial Transplant*, 13(Suppl 3): 9-11.
- **Masharani, U.and Michael, S. (2017).** Pancreatic hormonees and diabetes mellitus. In: Gardner, D and Shoback, D. (ed) Greenspan's basic and clinical endocrinology (9th ed). New York: McGraw-Hill Medical.:6 pp 941.
- **Massaro, E. J.and Rogers, J. M. (Eds) (2004).** The Skeleton: Biochemical, Genetic, and Molecular Interactions in Development and Homeostasis. *Springer Science and Business Media*.pp 422.

- **Mauvais-Jarvis ,F.; Sobngwi, E.; Porcher, R.; Riveline, J.P.; Kevorkian, J.P.; Vaisse, C.; Charpentier, G.; Guillausseau, P.J.; Vexiau, P. and Gautier, J.F (2004).** Ketosis-prone type 2 diabetes in patients of sub-Saharan African origin: clinical pathophysiology and natural history of beta-cell dysfunction and insulin resistance. *Diabetes* 53:645–653,
- **McCarthy, A. D.; Etcheverry, S. B.and Cortizo, A. M. (2001).** Effect of advanced glycation endproducts on the secretion of insulin-like growth factor-I and its binding proteins: role in osteoblast development. *Acta diabetological*, 38(3), 113-122.
- **Mera, P.; Laue, K .J.; Ferron, M.; Confavreux, C.; Wei, J.; Galan-Diez, M.; Lacampagne, A.; Mitchell, Sarah .; Mattison, J. A.; Chen, Y.; Bacchetta, J.; Szulc, P.; Kitsis, R. N. ; Cabo, R. D. ; Friedman, R.; Torsitano, C.; McGraw, T. E.; Puchowicz, M., Kurland, I. and Karsenty, G. (2016).** Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. *Cell Metabolism*, 23(6): 1078–1092.
- **Midtby, M.; Magnus, J. H.and Joakimsen, R. M. (2001).** The Tromsø Study: a population-based study on the variation in bone formation markers with age, gender, anthropometry and season in both male and women. *Osteoporosis international*, 12(10): 835-843.
- **Misra, S. and Hattersley, A.T. (2017).** Monogenic Causes of Diabetes. Textbook of Diabetes, 5th .Holt, R. ,Cockram, C., Flyvbjerg, A. and Goldstein, B., John Wiley and Sons, Ltd. Published by John Wiley and Sons, Ltd.243-258.
- **Mizokami, A.; Yasutake, Y.; Higashi, S.; Kawakubo-Yasukochi, T.; Chishaki, S.; Takahashi, I., Takeuchi, H. and Hirata, M. (2014).**Oral administration of osteocalcin improves glucose utilization by stimulating glucagon-like peptide-1 secretion. *Bone*, 69: 68-79.

- **Mizokami, A.; Kawakubo-Yasukochi, T.and Hirata, M. (2017).** Osteocalcin and its endocrine functions. *Biochemical pharmacology*, 132:1-8.
- **Mizokami, A.; Yasutake, Y.; Gao, J.; Matsuda, M.; Takahashi, I.; Takeuchi, H.and Hirata, M. (2013).** Osteocalcin induces release of glucagon-like peptide-1 and thereby stimulates insulin secretion in mice. *PloS one*, 8(2), e57375.
- **Moinuddin, K. and Awanti, S. M. (2016)**. Evaluation of the relationship between glycemic parameters and serum uric acid level in type 2 diabetes mellitus patients. *International Journal of Clinical Biochemistry and Research*. 3(4):395–401.
- **Moreira, C. A.; Barreto, F. C.and Dempster, D. W. (2015).** New insights on diabetes and bone metabolism. *Brazilian Journal of Nephrology*, 37(4): 490-495.
- **Mosekilde, L.; Vestergaard, P.and Rejnmark, L. (2013).** The pathogenesis, treatment and prevention of osteoporosis in male. *Drugs*, 73(1): 15-29.
- **Motyl, K. J.; McCabe, L. R.and Schwartz, A. V. (2010).** Bone and glucose metabolism: a two-way street. *Archives of biochemistry and biophysics*, 503(1):2-10
- **Mullur, R.; Liu, Y.Y. and Brent, G.A. (2014).** Thyroid hormone regulation of metabolism. *Physiological reviews*, *94*(2), 355-382.
- **Nagasaka, S.; Murakami, T.; Uchikawa, T.; Ishikawa, S. E.and Saito, T. (1995).** Effect Of Glycemic Control On Calcium And Phosphorus Handling And Parathyroid Hormone Level In Patients With Non-Insulin-Dependent Diabetes Mellitus. *Endocrine Journal*, 42(3): 377-383.
- **Nannipieri, M.; Cecchetti, F.; Anselmino, M.; Camastra, S.; Niccolini, P.; Lamacchia, M.; Iervasi, G.; Ferrannini, E. (2009).** Expression of thyrotropin

and thyroid hormone receptors in adipose tissue of patients with morbid obesity and/or type 2 diabetes: effects of weight loss. *International journal of obesity*, 33(9): 1001-1006.

- **Napoli, N.; Chandran, M.; Pierroz, D. D.; Abrahamsen, B.; Schwartz, A.V. and Ferrari, S. L. (2017).** Mechanisms of diabetes mellitus-induced bone fragility. *Nature Reviews Endocrinology*, 13(4): 208-219.
- **Napoli, N.; Strollo, R.; Paladini, A.; Briganti, S. I.; Pozzilli, P.; Epstein, S. (2014).** The alliance of mesenchymal stem cells, bone, and diabetes. *International journal of endocrinology*, 2014:1-26.
- **Natah, T. M.; Wtwt, M. A.; Al-Saadi, H. K.; Al-Saadi, A. H.and Farhood, H. F. (2013).** Study the levels of adiponectin, FSH, LH, and sex hormone in type 2 diabetes (NIIDDM). Journal of Biology, Agriculture and Health care, 3(2):172-181.
- **Ng, K. W.and Martin, T. J. (2009)**. New functions for old hormones: Bone as an endocrine organ. *Molecular and cellular endocrinology*, 1(310): 1-2.
- **Ngugi, M.P.; Njagi, J.M.; Kibiti, C.M.; Ngeranwa, J.J.N.and Njagi, E.N.M. (2012)**.Diagnosis of Diabetes Mellitus. *International Journal of Diabetes Research*, 1(2): 24-27.
- **Nilsson, L.; Binart, N.; Bohlooly-Y, M.; Bramnert, M.; Egecioglu, E.; Kindblom, J.; Kelly, P.A.; Kopchick, J. J; Ormandy, C.J; Lingx, C. and Billig, H. (2005)**. Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. *Biochemical and biophysical research communications*, 331(4): 1120-1126.
- **Njolstad, P.R.; Sagen, J.V.; Bjorkhaug, L.; Odili, S.; Shehadeh, N.; Bakry, D.; Sarici, S. U.; Alpay, F.; Molnes, J.; Molven, A.; Sovik, O. and Matschinsky, F. M. (2003)**.Permanent neonatal diabetes caused by glucokinase

deficiency: inborn error of the glucose-insulin signaling pathway. *Diabetes*, 52(11):2854-60.

- **Nna, V. U.; Bakar, A. B. A.and Mohamed, M. (2017).** Diabetes mellitus-induced male reproductive impairment: The role of natural products: A review. *Journal of applied pharmaceutical science*, 7(09): 233-242.
- **Ohe, M. N.; Santos, R. O.; Kunii, I. S.; Carvalho, A. B.; Abrahão, M.; Cervantes, O.; Lazaretti-Castro, M.and Vieira, J. G. H. (2003).** Usefulness of a rapid immunometric assay for intraoperative parathyroid hormone measurements. *Brazilian Journal of Medical and Biological Research*, 36(6): 715-721.
- **Onah, C.,Meludu, S., Dioka, C., Onuegbu, J., Amah U., Olisekodiaka, M., Okwara, J., Onah, C. and Ezeugwunne, I. (2013).** Pattern of male sex hormones in type 2 diabetic patients in Nnewi. *Journal of Medical Education.* 10: 65-70.
- **Oury, F.(2012).** A crosstalk between bone and gonads. *Annals of the New York Academy of Sciences* ,1260(1):1-7
- **Oury, F.; Ferron, M.; Huizhen, W.; Confavreux, C.; Xu, L.; Lacombe, J.;Srinivas P.; Chamouni, A.; Lugani, F.; Lejeune ,H. and Kumar, T. R. (2013).** Osteocalcin regulates murine and human fertility through a pancreas-bone-testis axis. *The Journal of clinical investigation*, 123(6): 2421-2433.
- **Oury, F.; Sumara, G.; Sumara, O.; Ferron, M.; Chang, H.; Smith, C. E., Hermo, L.; Suarez , S. S.; Roth, B. L.; Ducy, P. and Karsenty, G. (2011).** Endocrine regulation of male fertility by the skeleton. *Cell*, 144(5): 796-809.
- **Pasquel, F. J.and Umpierrez, G. E. (2014).** Hyperosmolar hyperglycemic state: a historic review of the clinical presentation, diagnosis, and treatment. *Diabetes care*, *37*(11), 3124-3131.

- **Paula, F. J. A.; Lanna, C. M. M.; Shuhama, T.and Foss, M. C. (2001).** Effect of metabolic control on parathyroid hormone secretion in diabetic patients. Brazilian Journal of Medicaland Biological Research, 34(9):1139-1145.
- **Pearson, D. A. (2007)** .Bone health and osteoporosis: the role of vitamin K and potential antagonism by anticoagulants. *Nutrition in Clinical Practice*, 22(5):517–544.
- **Peltier, A.; Goutman, S. A. and Callaghan, B. C. (2014).** Painful diabetic neuropathy. *Bmj*, *348*, g1799.
- **Petryk, A.; Fleenor, D.; Driscoll, P.and Freemark, M. (2000).** Prolactin induction of insulin gene expression: the roles of glucose and glucose transporter-2. *Journal of Endocrinology*, 164(3), 277-286.
- **Pi, M., Wu, Y. and Quarles, L. D. (2011)**. GPRC6A mediates responses to osteocalcin in β-cells in vitro and pancreas in vivo. *Journal of Bone and Mineral Research*, 26(7): 1680-1683.
- **Pi, M.; Chen, L.; Huang, M.; Zhu,W.; Ringhofer ,B.; Luo, J.; Christenson, L. K.; Li, B.; Zhang, J.; Jackson, P. D.; Faber, P. W.; Brunden, K, R.; Harrington, J.J. and Quarles, L .D. (2008).** GPRC6A null mice exhibit osteopenia,feminization and metabolic syndrome. *PLoS One*, 3(12): e3858.
- **Pi, M.; Kapoor, K.; Ye, R.; Nishimoto, S. K.; Smith, J. C.; Baudry, J.and Quarles, L. D. (2016)**. Evidence for osteocalcin binding and activation of GPRC6A in β-cells. *Endocrinology*, 157(5): 1866-1880.
- **Pittas, A. G.; Harris, S. S.; Eliades, M., Stark, P.and Dawson-Hughes, B. (2009).** Association between serum osteocalcin and markers of metabolic phenotype. *The Journal of Clinical Endocrinology and Metabolism*, 94(3): 827-832.

- **Pittas, A. G.; Lau, J.; Hu, F. B.and Dawson-Hughes, B. (2007).** The role of vitamin D and calciuml in type 2 diabetes. A systematic review and meta-analysis. *The Journal of Clinical Endocrinology and Metabolism*, 92(6): 2017-2029.
- **Pop-Busui, R.; Boulton, A. J.; Feldman, E. L.; Bril, V.; Freeman, R.; Malik, R. A.; Sosenko, J. M. and Ziegler, D. (2017)**. Diabetic neuropathy: a position statement by the American Diabetes Association. *Diabetes care*, *40*(1), 136-154.
- **Power, M. J.and Fottrell, P. F. (1991)**. Osteocalcin: diagnostic methods and clinical applications. *Critical reviews in clinical laboratory sciences*, 28(4): 287-335.
- **Powers, A. C. (2008)**. Diabetes mellitus. In: "Harrison, Brauwnwald, Kasper, editors. Harrison's Principles of Internal Medicine 17th ed." McGraw-Hill Companies, Inc; pp. 2275–2304.
- **Pugliese, A. and Eisenbarth, G. S. (2007).** Human Type 1 Diabetes Mellitus: Genetic Susceptibility and Resistance. In Type1 Diabetes: Molecular, Cellular, and Clinical Immunology. 3rd ed., G.S. Eisenbarth, K.J. Lafferty. New York. 134-152.
- **Punthakee, Z.; Goldenberg, R. and Katz, P. (2018)**. Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome.Canadian Journal of Diabetes 42: S10–S15.
- **Puspitasari, M. D.; Purnamasari, D.; Setyohadi, B. and Isbagio, H. (2017).** Bone metabolism and fracture risk in diabetes mellitus. *Journal of the ASEAN Federation of Endocrine Societies*, 32(2): 90-99.

- **Qatanani, M.and Lazar, M. A. (2007).** Mechanisms of obesity-associated insulin resistance: many choices on the menu. *Genes and development*, 21(12): 1443-1455.
- **Razzaque, M. S. (2011).** Osteocalcin: a pivotal mediator or an innocent bystander in energy metabolism?. *Nephrology Dialysis Transplantation*, 26(1): 42-45.
- **Risérus, U.; Willett, W. and Hu, FB. (2009).** Dietary fats and prevention of type 2 diabetes. *Progress in lipid research*, 48(1): 44–51.
- **Robert, A.V.; Amy, F.; Allan, R. (2006).** Glass, Thyrotropin Suppression by Metformin, *The Journal of Clinical Endocrinology and Metabolism*, 91(1): 225–227,
- **Robling, A. G.; Castillo, A. B. and Turner, C. H. (2006).** Biomechanical and molecular regulation of bone remodeling. *Annual Review of Biomedical Engineering,* 8(1): 455–498.
- **Roelfsema, F.; Pijl, H.; Keenan, D. M.and Veldhuis, J. D. (2012).** Prolactin secretion in healthy adults is determined by gender, age and body mass index. *PloS one*, *7*(2).
- **Røislien, J.; Van Calciumlster, B.and Hjelmesæth, J. (2011).** Parathyroid hormone is a plausible mediator for the metabolic syndrome in the morbidly obese: a cross-sectional study. *Cardiovascular diabetology*, 10(1): 1-8
- **Rosner, W.; Auchus, R. J.; Azziz, R.; Sluss, P. M.and Raff, H. (2006).** Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *The Journal of Clinical Endocrinology and Metabolism*, 92(2): 405-413.
- **Rother, K. I. (2007).** Diabetes treatment—bridging the divide. *The New England journal of medicine*, 356(15):1499.

- **Rubin, M.R. (2015).** Bone cells and bone turnover in diabetes mellitus.*Current Osteoporosis Reports*, 13(3):186–191.
- **Sabek, O. M.; Nishimoto, S. K.; Fraga, D.; Tejpal, N.; Ricordi, C.and Gaber, A. O. (2015).** Osteocalcin effect on human β-cells mass and function. *Endocrinology*, 156(9): 3137-3146.
- **Saltiel, A.R. and Kahn, C.R. (2001).** Insulin signalling and the regulation of glucose and lipid metabolism. *Nature*. 414(6865):799-806.
- **Schwartz, A. V.and Sellmeyer, D. E. (2004).** Women, type 2 diabetes, and fracture risk. Current diabetes reports, 4(5): 364-369.
- **Schwarz, P.; Sørensen, H. A.; Momsen, G.; Friis, T.and McNair, P. (1992).** Hypocalcemia and parathyroid hormone responsiveness in diabetes mellitus: a tri-sodium-citrate clamp study. *European Journal of Endocrinology*, 126(3): 260-263.
- **Schwetz, V. (2013).**Ph.D.thesis" The Endocrine Role of the Skeleton – Osteocalcin, Glucose Metabolism and Fertility Endocrinol. Division of Endocrinology and Metabolism, Department of Internal Medicine, Medical University of Graz, Auenbruggerplatz 15, Graz, Austria
- **Shanbhogue, V. V.; Mitchell, D. M.; Rosen, C. J.and Bouxsein, M. L. (2016).** Type 2 diabetes and the skeleton: new insights into sweet bones. The lancet Diabetes and endocrinology, 4(2): 159-173.
- **Shao, J.; Wang, Z.; Yang, T.; Ying, H.; Zhang, Y.and Liu, S. (2015).** Bone regulates glucose metabolism as an endocrine organ through osteocalcin. *International Journal of Endocrinology*, 2015(3):1-9
- **Shlomo, M.; Kenneth, P.; Reed, L. and Henry, K. (2011).**Williams textbook of endocrinology.(12th ed.). Philadelphia: Elsevier/Saunders. pp. 1371–1435

- **Shulman, G. I. (2000).** Cellular mechanisms of insulin resistance. *The Journal of clinical investigation*, 106(2): 171-176.
- **Smith-Morris, C. (2005).** Diagnostic controversy: Gestational diabetes and the meaning of risk for Pima Indian women. *Medicine Anthropology*. 24: 145-177.
- **Spitzer, M.; Basaria, S.; Travison, T. G.; Davda, M. N.; DeRogatis, L.and Bhasin, S. (2013).** The effect of testosterone on mood and well-being in male with erectile dysfunction in a randomized, placebo-controlled trial. Andrology, 1(3): 475-482.
- **SPSS, (2015).** Statistical package for Social Science, Users Guide for Statistics.
- **Stafford, D.W (2005).** The vitamin K cycle. *Journal of Thrombosis and Haemostasis* 3(8):1873-1878.
- **Starr, J. I.; Mako, M. E.; Juhn, D.and Rubenstein, A. H. (1978).** Measurement of serum proinsulin-like material: cross-reactivity of porcine and human proinsulin in the insulin radioimmunoassay. *The Journal of laboratory and clinical medicine*, 91(4): 683-692.
- **Starup-Linde, J.; Westberg-Rasmussen, S.; Lykkeboe, S.; Handberg, A.; Hartmann, B.; Holst, J. J.; Hermansen, K.; Vestergaard, P.and Gregersen, S. (2018).** Glucose tolerance tests and osteocalcin responses in healthy people. *Frontiers in endocrinology*, 9: 356.
- **Starup-Linde, J.and Vestergaard, P. (2015).** Management of endocrine disease: diabetes and osteoporosis: cause for concern?. *European journal of endocrinology*, 173(3): R93-R99.
- **Steffens, J. P.; Herrera, B. S.; Coimbra, L. S.; Stephens, D. N.; Rossa Jr, C.; Spolidorio, L. C.; Kantarci,A. and Van Dyke, T. E. (2014).** Testosterone

regulates bone response to inflammation. *Hormone and Metabolic Research*, 46(3): 193-200.

- **Stern, P.H.(1996).** Thyroid hormone and bone. In: Principles of Bone Biology. Bilezikian ,J.P.; Raisz ,L.G. and Rodan, G.A. (eds). Academic Press, San Diego,521-531.
- **Strotmeyer, E. S.and Cauley, J. A. (2007).** Diabetes mellitus, bone mineral density, and fracture risk. *Current Opinion in Endocrinology, Diabetes and Obesity*, 14(6): 429-435.
- **Szulc, P.; Kaufman, J.M.and Delmas, P.D.(2007).**Biochemical assessment of bone turnover and bone fragility in male. *Osteoporosis International* 18(11):1451-1461.
- **Tayde, P.; Borle, A.; Zanwar, Y.; Rode, M.and Phatak, M. (2013).** Glycated hemoglobin pattern and its correlation with lipid profile in type-2 diabetic males in central India. *National J of Community Medicine*, 4(4): 564-568.
- **Teitelbaum, S. L.and Ross, F. P. (2003).** Genetic regulation of osteoclast development and function. *Nature Reviews Genetics*, 4(8): 638.–649.
- **Thomas L. (1998)**.Parathyroid hormone (PTH) Clinical Laboratory Diagnosis.TH-Books, Frankfurt. 1st english edition: 248-250.
- **Tietz, N.W. (1995)**. Clinical Guide To Laboratory Tests. 3rd ed. Philadelphia, Pa:WB Saunders Co, 410.
- **Tietz, N.W. (2006).**Clinical Guide to Laboratory Tests, 4th ed. Philadelphia:WB Saunders Co 2006:444-451.
- **Tirupathi, C.; Suryanarayana, B.; Siddeswari, R.; Sudarsi, B. and Manohar, S. (2015)**. A study of Correlation of Body Mass Index, Waist Hip

Ratio and Lipid Profile in Type II Diabetes Mellitus Subjects. *IJSRP*. 5(4): 2250-3153.

- **Vamos, E. P.; Millett, C.; Parsons, C.; Aylin, P.; Majeed, A.and Bottle, A. (2012).** Nationwide study on trends in hospital admissions for major cardiovascular events and procedures among people with and without diabetes in England, 2004–2009. *Diabetes care*, *35*(2), 265-272.
- **Vikhe, V.B.; Kanitkar, S.A.; Tamakuwala, K.K.; Gaikwad, A.N.; Kalyan, M. and Agarwal, R.R. (2013).**Thyroid dysfunction in patients with type 2 diabetes mellitus at tertiary care center". National Journal of medical research; 3(4):377-380.
- **Villafan-Bernal, J.R.; Sanchez-Enriquez, S. and Munoz-Valle, J.F. (2011).** Molecular modulation of osteocalcin and its relevance in diabetes (review). *International Journal of Molecular Medicine* 28(3): 283-293.
- **Vital, P.; Larrieta, E.and Hiriart, M. (2006).** Sexual dimorphism in insulin sensitivity and susceptibility to develop diabetes in rats. *Journal of Endocrinology*, 190(2): 425-432.
- **Wang, J.S.; Lee, I.T.; Lee, W.J.; Lin, S.Y.; Fu, C.P.; Ting, C.T.; Lee, W.L.; Liang, K.W and Sheu, W.H.H. (2013)** .Performance of HbA1c and fasting plasma glucose in screening for diabetes in patients undergoing coronary angiography. *Diabetes Care*, 36(5):1138-1140.
- **Wei, J.; Hanna, T.; Suda, N.; Karsenty, G.and Ducy, P. (2014).** Osteocalcin promotes β-cell proliferation during development and adulthood through Gprc6a. *Diabetes*, 63(3): 1021-1031.
- **Wild, D. (Ed.). (2013).** The immunoassay handbook: theory and applications of ligand binding, ELISA and related techniques. Elsevier Science ,pp 1036

- **Wild, D.(1994).**The immunoassay handbook. Macmillan ; New York,pp 83-100
- **Wolfsdorf, J.I.; Allgrove ,J.; Craig, M.E.; Edge, J.; Glaser, N.; Jain, V.; Lee, W.W.; Mungai, L.N.; Rosenbloom, A.L.; Sperling, M.A and Hanas, R. (2014)** International Society for Pediatric and Adolescent Diabetes: ISPAD Clinical Practice Consensus Guidelines 2014. Diabetic ketoacidosis and hyperglycemic hyperosmolar state. *Pediatr Diabetes* 15(Suppl 20):154–179.
- **World Health Organization (WHO) (2019).** Mean Body Mass Index (BMI). Retrieved 5 February 2019.
- **World Health Organization. (1999).** Definition, diagnosis and classification of diabetes mellitus and its complications: report of a WHO consultation. Part 1, Diagnosis and classification of diabetes mellitus (No. WHO/NCD/NCS/99.2). Geneva: World health organization.
- **Wu, A. H. (2006)**. Tietz clinical guide to laboratory tests 4th Edition .Elsevier Health Sciences. 412 pp.
- **Xu, F.; Dong, Y.; Huang, X.; Li, M.; Qin, L.; Ren, Y.Guo, F.; Chen, A. and Huang, S. (2014).**Decreased osteoclastogenesis, osteoblastogenesis and low bone mass in a mouse model of type 2 diabetes. *Molecular medicine reports*, 10(4): 1935-1941.
- **Yamaguchi, T.and Sugimoto, T. (2011).** Bone metabolism and fracture risk in type 2 diabetes mellitus. *Endocrine journal*, 1107160591-1107160591.
- **Yamamoto, M.; Yamaguchi, T.; Nawata, K.; Yamauchi, M.and Sugimoto, T. (2012).** Decreased PTH levels accompanied by low bone formation are associated with vertebral fractures in postmenopausal women with type 2 diabetes. *The Journal of Clinical Endocrinology and Metabolism*, 97(4): 1277-1284.

- **Yang, J.; Zhang, X.; Wang, W.and Liu, J. (2010).** Insulin stimulates osteoblast proliferation and differentiation through ERK and PI3K in MG-63 cells. Cell Biochemistry and Function: Cellular biochemistry and its modulation by active agents or disease, 28(4): 334-341.
- **Yeap, B.B.; Chubb, S.A.; Flicker, L.; McCaul, K.A.; Ebeling, P.R.; Beilby, J.P.and Norman, P.E. (2010).**Reduced serum total osteocalcin is associated with metabolic syndrome in older male via waist circumference, hyperglycemia, and triglyceride levels. *European Journal of Endocrinology*, 163:265-272.
- **Yu, S.; Franceschi, R. T.; Luo, M.; Zhang, X.; Jiang, D.; Lai, Y. ; Jiang, Y., Zhang, J. and Xiao, G. (2008).** Parathyroid hormone increases activating transcription factor 4 expression and activity in osteoblasts: requirement for osteocalcin gene expression. *Endocrinology*, 149(4): 1960-1968.
- **Zakeri, Z.; Azizi, Z.; Mehrabifar, H.and Hashemi, M. (2011).** Evaluation of bone mineral density in premenopausal women with type-2 diabetes mellitus in Zahedan, southeast Iran. *Journal of the Pakistan Medicall Association*, 61(5): 443.
- **Zanatta, L. C.; Boguszewski, C. L.; Borba, V. Z.and Kulak, C. A. (2014).** Osteocalcin, energy and glucose metabolism. *Arquivos Brasileiros de Endocrinologia and Metabologia*, 58(5), 444-451.
- **Zeitlin, S. I.and Rajfer, J. (2000).** Hyperprolactinemia and erectile dysfunction. *Reviews in urology*, 2(1): 39.
- **Zoch, M. L.; Clemales, T. L. and Riddle, R. C. (2016)**. New insights into the biology of osteocalcin. *Bone*, 82: 42-49.

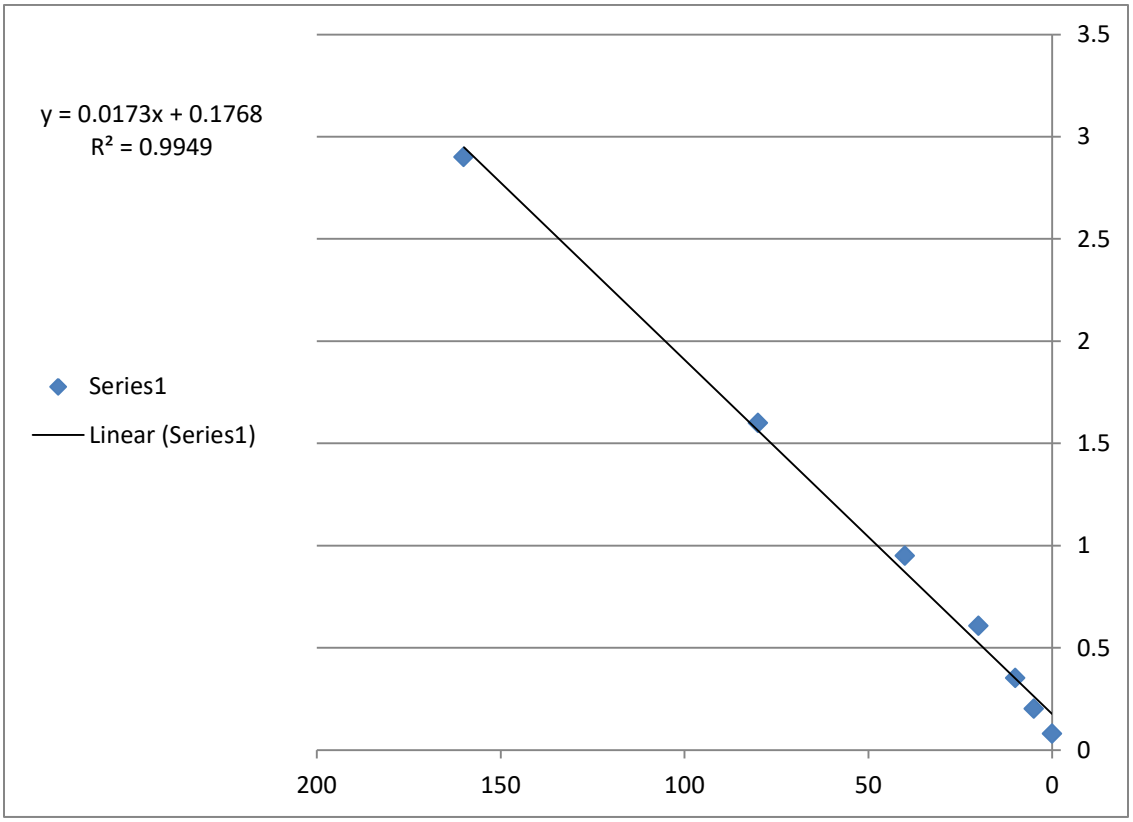
Appendixes

Appendixes

Questionnaire

Calciumse No.	
Age	
weight	
height	
Does he smoking	
Are you suffer from(calciumrdiac disease, hypertension, acute and chronic infections, renal disease, hepatic dysfunction, bone disease	
Blood examination	
HbA1c	
Serum examination	
FBG	
Calcium	
OC	
IN	
LH	
FSH	
TT	
PRL	
T3	
T4	
TSH	
PTH	

Appendix (1)


y = 0.0173x + 0.1768
R² = 0.9949
Series1
Linear (Series1)
3.5
3
2.5
2
1.5
1
0.5
0
200
150
100
50
0

Appendix (2)

الخلاصة

هدفت الدراسة الحالية إلى تقييم والتحقق من العلاقة بين الاوستيوكالسين (OC) وبعض الهرمونات والمعايير الكيميائية الحيوية لدى مرضى ذكورمصابين بداء السكري من النوع الثاني في عينة من الذكور في محافظة ميسان خلال الفترة من كانون الاول 2018 إلى ايار 2019 وشملت الدراسة 110 ذكور (66 مصابين بمرض السكري من النوع الثاني و 44 أصحاء) الذين تتراوح أعمارهم بين 35 الى 49 سنه.

تم تقسيم عينة الدراسة حسب العمر إلى ثلاث مجموعات ، الأولى (35-39) سنة ، والثانية (40-44) سنة والثالثة (45-49) سنة. أيضًا قسمت العينه وفقًا لمؤشر كتلة الجسم (BMI) إلى ثلاث مجموعات ، الوزن الطبيعي (18.5-24.9) ، زيادة الوزن (25-29.9) والسمنة (BMI> 30) . أظهرت نتائج هذه الدراسة أن قيم سكر الصيام (FBG) ،السكر التراكمي (HbA1c) ، الأوستيوكالسين (OC) والأنسولين في المرضى الذين يعانون من T2DM ازدادت معنويا (P<0.05)عند المقارنة مع مجاميع السيطرة، بينما قيم البرولاكتين في مرضى السكري انخفضت معنويا (P <0.05) عند المقارنة مع مجموعة السيطرة. زادت قيم FBG و HbA1c معنويا في مجموعات مرضى السكري مقارنتة مع مجموعات السيطرة لجميع الفئات العمرية ومؤشر كتلة الجسم.

ارتفعت قيم الكالسيوم الكلي (Ca) في مرضى السكري في المجموعه العمريه الثانيه ومجموعة الوزن الزائد معنويا (P <0.05) مقارنته بمجاميع السيطرة. أظهرت قيمة هرمون OC زيادة معنويه (P <0.05) في مرضى المجموعه العمرية الثانية عند مقارنتها بمجموعه السيطره ومرضى المجموعه العمرية الأولى ، كما ارتفعت قيم OC معنويا (P <0.05) في المرضى الذين يعانون من زيادة الوزن و السمنة مقارنة بمجموعة السيطرة لنفس المجاميع. ارتفعت قيمة الأنسولين معنويا (P <0.05)في المجموعة العمرية الأولى ، الوزن الطبيعي والسمنة مقارنته بالسيطرة لنفس المجاميع ، ارتفعت قيمة الأنسولين معنويا (P <0.05) في مرضى السمنة مقارنته بالمرضى في المجاميع الاخرى.

أظهرت النتائج أن قيم الهرمون اللوتيني (LH) في مجموعة العمرالثالثة ومجموعة الوزن الزائد ارتفعت معنويا (P <0.05) مقارنته بالسيطرة في نفس المجموعات ، لكن قيم LH و TT اختلفت

معنويا بين مرضى المجاميع العمرية. في حين انخفضت قيمة البرولاكتين (PRL) معنويا في مجموعة العمر الأولى ومجموعة الوزن الطبيعي في مرضى السكري مقارنته بمجموعات السيطره. لم تختلف قيم هرمونات الغدة الدرقية وهرمون محفز الغدة الدرقية بين المرضى ومجاميع السيطرة لجميع الفئات العمرية ومؤشر كتلة الجسم. انخفضت قيمة هرمون جار الدرقية (PTH) معنويا في مرضى مجموعة العمر الأول ومجموعة الوزن الطبيعي مقارنته بالسيطرة لنفس المجموعات.

بالنسبة للارتباط ،لم تظهر النتائج أي علاقة بين OC والمعايير المدروسه اعلاه. استنتجت الدراسة الحالية إلى أن مستويات OC والأنسولين و PTH تزداد مع تقدم العمر و زياده مؤشر كتلة الجسم ، بينما انخفض تركيزPRL في مرضى السكر من النوع الثاني.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة ميسان/ كلية العلوم

قسم علوم الحياة

تقييم مستوى الأوستيوكالسين وبعض الهرمونات والمعايير الكيموحيوية لدى الذكورالمرضى المصابين بمرض السكري من النوع الثاني في محافظة ميسان

رسالة مقدمة الى

مجلس كلية العلوم / جامعة ميسان

وهي جزء من متطلبات نيل درجة الماجستير علوم في علوم الحياة

من قبل

ايمان علي حسين الساعدي

بكالوريوس علوم /علوم الحياة (2013)

بأشراف **أ.د زينب عبد الجبار رضا العلي**

ايلول ٢٠١٩ م **محرم ١٤٤١ هـ**