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A Comparative study between thyroid hormones and lipid profile in diabetes Type 2

A Thesis

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By

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إهــداء

الى من كلله الله بالهيبة والوقار الى من علمني العطاء بدون انتضار الى المربي الفاضل ....

أبي الغالي

الى من علمتني الصمود مهما تبدلت الظروف الى من كان دعائها سر نجاحي الى نبع المحبة والحنان .....

أمي الغاليه

الى رفيق دربي الى من سار معي نحو الحلم خطوة بخطوة ..... زوجي الغالي

الى سندي وملاذي بعد الله ..... اخوتي

الى من ارى التفاؤل بعينها والسعاده في ضحكتها ..... ابنتي

الى كل من وقف بجانبي ومد يد العون .....

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نور

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Noor

Summary

This study focused on the survey of thyroid abnormalities symptoms (hypo and hyperthyroidism) and estimating the level of these hormones free tri-iodothyronine (FT3), free tetra-iodothroxine (FT4) and thyroid stimulating hormone (TSH) among type 2 diabetic Iraqi patients. Also the lipid profile triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels for these diabetic patients were compared with non diabetic healthy group. Ninety Cases in this study were consist of thirty diabetic patients with hypothyroidism and another thirty diabetic patients with hyperthyroidism. Thirty blood samples from healthy non diabetic and non endocrine disease people were chosen as controls. Complete information includes: history of diabetes, age, sex, length, weight, body mass index and any other disease were taken for each case under study. The result showed high incidence of abnormal thyroid hormones level were observed in type 2 diabetic patients, where the level of FT3 in diabetic patients with hyperthyroidism was(5.35) µL/mL, while in diabetic patients with hypothyroidism was (4.48) µL/mL compared with healthy control (5.79) µL/mL. The level of FT4 in diabetic patients with hyper and hypothyroidism (14.94, 15.08) µL/mL respectively, compared with healthy control (15.07) µL/mL. While the level of TSH in diabetic patients with hyper and hypothyroidism (0.96,7.21)µL/mL respectively, compared with healthy control (1.88) µL/mL . Also the results of statistical analysis showed a significant correlation in at least at $P<0.05$ between total cholesterol, triglyceride with FT3 and TSH in hypo and hyperthyroidism. On the other hand, the results showed a significant correlation at $P<0.05$ in age and body mass index in diabetic patients when compared with control group. Also statistical analysis showed that thyroid disorders were more prevalent in female than male.

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Acc	Acetyl COA carboxylase
ADH	Alcohol dehydrogenase
AITD	Autoimmune thyroid disease
DIT	Diiodothyrosine
DM	Diabetes mellitus
FAS	Fatty acid synthase
FT3	Free triiodothyronine
FT4	Free thyroxin
GD	Graves' disease
GLUT4	Translocation of glucose transporters
HDL	High density lipoprotein
HSL	Hormone sensitive lipase
HT	Hashimoto's thyroiditis
IDDM	Insulin-dependent diabetes mellitus
LCAT	Lecithin cholesterol acyltransferase
LDL	Low density lipoprotein
LPL	Lipoprotein lipase
MHC	Major histocompatibility complex
MIT	Monoiodotyrosine
NE	Norepinphrine
NIDDM	Non insulin-dependent diabetes mellitus
PPT	Postpartum thyroiditis

RER	Rough endoplasmic reticulum
SCH	Subclinical hyperthyroidism
SPR	Solid phase recepticale
T3	Triiodothyronine
T4	Thyroxin
TAO	Thyroid associated orbitopathy
TBG	Thyroxin-binding globulin
TBPA	Thyroxin-binding prealbumin
TH	Thyroid hormones
TPO	Thyroid peroxidase
TRH	Thyroid releasing hormone
TSH	Thyroid stimulating hormone
TSH-R	Thyroid stimulating hormone receptor
VLDL	Very low density lipoprotein

Chapter one

Introduction

and

Literatures Review

1.1 Introduction

Diabetes mellitus is a group of metabolism disease characterized by high blood sugar, since the body does not produce enough insulin, or because cells do not respond to the insulin that is produced. This high blood sugar produces the classical symptoms of polydipsia (increased thirst) , polyuria (frequent urination) leading and (increased hunger) polyphagia (Charels, 2009). Diabetes mellitus cause of death worldwide, is one of the most challenging health problems in 21st century (Gurjeet *et al*., 2011).

Thyroid gland is one of the important organs in human body that produces important hormones: T3 (triiodothyronine) and T4 (tetra-iodothyroxine) which have an important role in regulation of metabolic functions, growth and development (Karnath and Hussain, 2006). Hypothyroidism (Hashimotos' thyroiditis) and hyperthyroidism (Graves' disease) are the most common autoimmune thyroid disorders as one of most complications of thyroid dysfunctions. On the other hand, autoimmune disease occur when immune system begins to attack its own self antigens, so that the best feature of autoimmune thyroid disease is the presence of auto-antibodies against thyroid antigens (Lazurova and Benhatchi, 2012).

Diabetes mellitus and thyroid diseases are the two common endocrinopathies seen in adults population. Thyroid disorder and diabetes mellitus have been shown to mutually influence each other . The first reports showing the association between diabetes and thyroid dysfunction were published in 1979 . Thyroid hormones directly control insulin secretion. In hypothyroidism, there is a reduction in glucose-induced insulin secretion by beta cells, and the response of beta cells to glucose is increased in hyperthyroidism due to increased beta cell mass. Moreover, insulin clearance is increased in thyrotoxicosis. Diabetes may affect the thyroid function to variable extent. Diabetes mellitus

appears to influence thyroid function in two sites; first at the level of hypothalamic control of TSH release and second at peripheral tissue by converting T4 to T3.

This study was aimed to study thyroid disorders in diabetic type 2 patients and compare them with results obtained from a sample of the normal adult population and relation between diabetes and thyroid dysfunction. This study suggests that thyroid function should be screened annually in diabetic type 2 patients to detect asymptomatic thyroid dysfunction which is increased in frequency in diabetic patient. On the other hand, lipid profile and C-peptide were measured to determined the relationship between them and thyroid disorders in diabetic type 2 patients.

Objective:

1. Studying the effect of hypothyroidism and hyperthyroidism on diabetes in a sample of Iraqi patients.

2. Investigating the relationship between lipid profile and thyroid function in diabetic patients.

1.2 Literature Review

1.2.1 Insulin

Insulin is a hormone play a critical role in balancing glucose levels in the body; human insulin has a molecular weight of 5808 and consist of two amino acid chains, A-chain and B-chain which are linked together by disulfide bonds (David and Dolores, 2011). Raymond (2010) reported that insulin is synthesized in the pancreas within the B-cells of the islets of Langerhans. It is however first synthesized as a single polypeptide called preproinsulin (115 amino acids) in pancreatic B-cells as shown in figure (1-1). Preproinsulin contains a 24_residue signal peptide which directs the nascent polypeptide chain to the rough endoplasmic reticulum (RER). The signal peptide is cleaved as the polypeptide is translocated into lumen of RER, forming proinsulin (85 amino acids). Proinsulin undergoes maturation into active insulin (51amino acids) through the action of cellular endopeptidases (Guyton and Hall, 2006).While Potter and Wilkin (2000) mentioned that the endopeptidases cleave at 2 position, releasing a fragment called the C-peptide, and leaving 2 peptide chains, the B and A chains, linked by 2 disulfide bonds. The C-peptide is the central portion of proinsulin, and the primary sequence of proinsulin goes in order "B-C-A". However, Beta cells in the islets of Langerhans release insulin in two phases: The first phase release is rapidly triggered in response to increased blood glucose levels and the second phase is a sustained slow release of newly formed vesicles triggered independently of sugar.

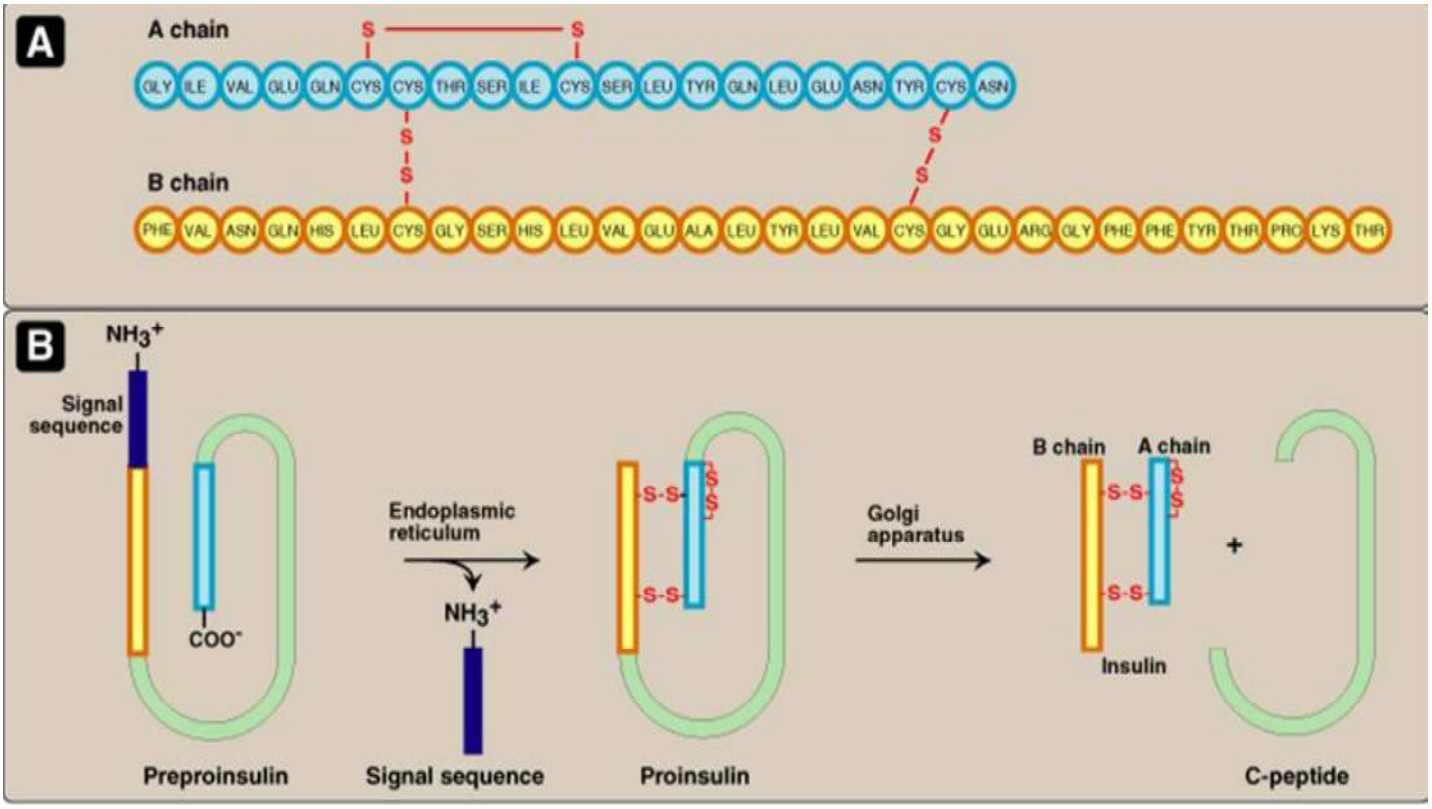

Figure (1.1): A. Structure of insulin. B. Formation of human insulin from preproinsulin (Potter and Wilkin, 2000)

1.2.1.1 Insulin receptors

The insulin receptors is a large transmembrane glycoprotein found in insulin sensitive target cells (liver, muscle and fat) (Ward *etal*., 2008). It comprises two extracellular R-subunits that contain the insulin-binding domain and two membrane spanning subunits that contain a ligand activated tyrosine kinase , which will be referred to as the insulin receptor tyrosine kinase. Insulin acts by binding to the extracellular domain of the insulin receptor, thus inducing autophosphorylation and activation of the insulin receptor tyrosine kinase. On the other hand, a cascade of signaling events is initiated leading to increased tyrosine phosphorylation of multiple intracellular substance, including the insulin receptor substrates 1 and 2, and the activation of second messenger systems such as phosphatidylinositol 3- kinase (White,

2008). Figure (1-2) illustrates these pathways act to trigger the translocation of glucose transporters 4(GLUT4) to the cell surface and GLUT4 is one of a family of membrane proteins responsible for glucose uptake in mammalian cells and is the major isoform responsive to insulin stimulation (Huang and Czech, 2007).

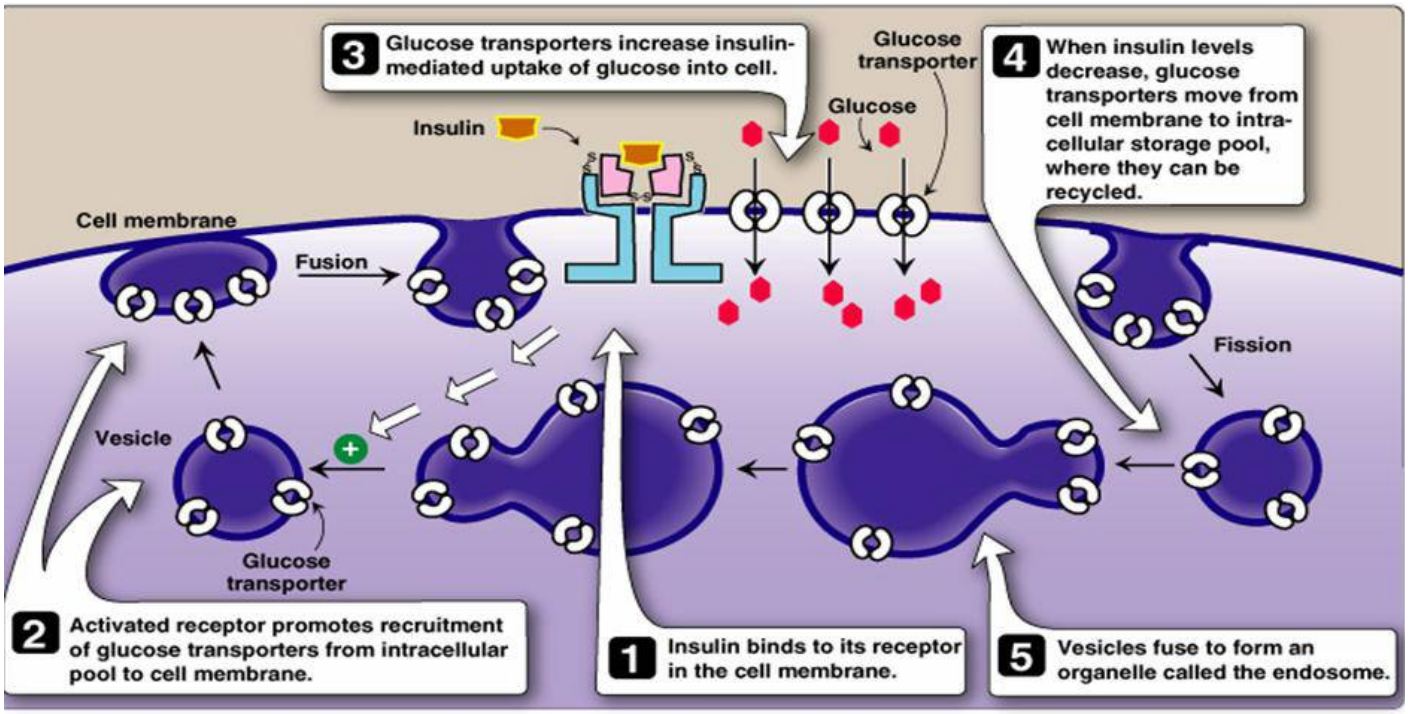

Figure (1.2) Insulin causes cells to recruit transporters from intracellular stores (Huang and Czech, 2007).

1.2.1.2 Mechanism of action of insulin

The net effect of insulin is to enhance storage and block mobilization and oxidation of fatty acids and insulin exerts its effect by stimulating lipoprotein lipase (LPL) formation, so that circulating triglycerides are hydrolyzed and fatty acid can enter the adipocyte. Researchers noticed that, insulin is also required for the transport of glucose , which is needed for re-esterification of the triglycerides once inside the adipocyte. Finally, the conversion of glucose to fatty acids is accomplished by insulin's activation of several enzymes (Guyton, 2006). Lipolysis is the chemical decomposition and release of fat from adipose tissue. This process predominates over lipogenesis when additional energy is required and the triglycerides within the

adipocyte are acted upon by a multi-enzyme complex called hormone sensitive lipase (HSL), which hydrolyzes the triglyceride into free fatty acids and diglycerides and monoglycerides. On the other hand, insulin reduces mobilization of fatty acids from adipose tissue by inhibiting triglyceride lipase. The mechanism of this inhibition may be through a decrease in cyclic AMP which in turn results in an inhibition of cyclic-AMP-dependent protein kinase (Albright and Stem, 1998).

1.2.1.3 Insulin resistance

Resistance is described that cells fail to respond to the normal actions of the insulin hormone that lead to high blood sugar, on the other hand Beta cells in the pancreas increase their production of insulin, and contributing to a high blood insulin level. This often remains undetected and can contribute to a diagnosis of type 2 diabetes (Thomas, 1993). Ivanova *et al*. (2009) reported that insulin's action on lipid metabolism is analogous to its role in glucose metabolism, promoting anabolism and inhibiting catabolism. Specifically, insulin upregulates LPL and stimulates gene expression of intracellular lipogenic enzyme, such as fatty acid synthase (FAS) and acety COA carboxylase (ACC) . In addition, insulin inhibits adipocyte HSL through inhibition of its phosphorylation (Anthonsen *etal*., 1998). In the insulin resistant state, the responses of both LPL and HSL to insulin are blunted . Thus, with insulin resistance, inefficient trapping of dietary energy occurs both because of decreased LPL-mediated lipolysis of chylomicrom-TAG and ineffective inhibition of HSL-mediates lipolysis in adipose tissue (Coppack *etal.*, 1992) . On the other hand, Dimitriadis *et al*. (2011) mentioned that the increased flux of fatty acids in blood stream effect on insulin sensitivity and lead to accumulation of (TAG) in glucose metabolizing tissues such as liver, skeletal muscle and pancreatic B-cell. Also, the accumulation of TAG in these tissues by unknown mechanism, but probably involving local TAG hydrolysis and availability of fatty acids lead to an impairment of the normal sensitivity of glucose metabolism to insulin.

1.2.2 Diabetes mellitus

Diabetes mellitus is a common endocrine disorder, it is the leading cause of adult blindness, amputation, and a major cause of renal failure, heart attack and stroke. Diabetes is not a one disease but rather is a heterogeneous group of syndromes characterized by an elevation of fasting blood glucose caused by a relative or absolute deficiency in insulin, metabolic alterations is caused by inadequate release of insulin are aggravated by an excess of glucagon (Abdelgader et *al.*, 2006). Hyperglycemia has an important role in the pathogenesis of long-term complication. Diabetic patients with poor blood glucose control are particularly at risk. Furthermore, complications appear to affect organs where cell do not require insulin for glucose uptake, such as those of the nervous system, heart, kidneys and small blood vessels. Consequently, these cells have high concentrations of intracellular glucose during hyperglycemia (Nessar et *al.*, 2004). Diabetes is characterized by polydipsia, polyuria, polyphagia, coma, ketosis and acidosis. There are wide spread biochemical abnormalities but the fundamental defects to which most of the abnormalities can be traced are reduced entry of glucose into the circulation from liver. There is therefore an extra cellular glucose excess and in many cell, an intracellular glucose deficiency, there is also decrease in the entry of amino acids into muscle and an increase in lipolysis (Ganong, 2003).

1.2. 3 Classification of diabetes mellitus

The World Health Organization classified diabetes mellitus in to groups:

1. Insulin_ dependent diabetes mellitus (IDDM).
2. Non insulin_ dependent diabetes mellitus (NIDDM).
3. Gestational diabetes.
4. Other specific types.

This division is important clinically in assessing the need for treatment and in understanding the causes of diabetes (knip, 1997 and Lenoid, 2010).

1.2.3.1 Insulin dependent diabetes mellitus (IDDM)

Diabetes mellitus type 1 is characterized by deficiency of insulin that result from autoimmune destruction of insulin_ producing beta cells of the pancreas. Destruction progresses sub clinically over months or years until beta cell mass decreases to the point that insulin concentrations are no longer adequate to control plasma glucose levels. This destruction result stimulus from susceptibility genes, auto antigens and environmental factors. Susceptibility genes are more common among some populations than among others and explain the higher prevalence of type 1 DM in some ethnic groups (Scandinavians, Sardinians), auto antigens include glutamic acid decarboxylase, insulin, proinsulin, and other proteins in beta cells. It is thought that these proteins are exposed or released during normal beta cell turnover or beta cell injury, activating primarily a T cell-mediated immune response resulting in beta cell destruction (insulitis). Glucagon secreting α cells remain unharmed. Antibodies to auto antigens, which can be detected in serum, seem to be a response to beta cell destruction. Finally, several viruses including rubella virus , retroviruses and cytomegalovirus have been linked to onset of type 1 DM. Viruses may directly infect and destroy beta cell, or they may cause beta cell destruction indirectly by exposing auto antigens, activating auto reactive lymphocytes mimicking molecular sequences of auto antigens that stimulate an immune response, or other mechanisms. Type 1 diabetes making about 5-10% of those diabetes and can affect children or adults. However, symptoms of IDDM appear abruptly when 80% to 90% of B-cells have been destroyed (Rother , 2007) . At this point the pancreas fails to respond adequately to ingestion of glucose . On the other hand, patients with type 1 diabetes can usually be recognized by appearance of polyphagia (excessive hunger), polyuria (frequent urination), fatigue, weight loss and weakness and the diagnosis is confirmed by fasting blood glucose greater than 140mg/dl (Royand and Lloyd , 2012). Diabetic mellitus type 1 is fatal unless treated with insulin. Injection is the most common method of

administering insulin.

Pancreas and islet transplants have been used to treat type 1 diabetes; however, islet transplants are currently still at the experimental trial stage (Philip *et al*, 2000).

1.2.3.2 Non insulin_ dependent diabetes mellitus (NIDDM)

Non insulin diabetes mellitus or type 2 diabetes mellitus is characterized by hyperglycemia which is result from a combination of defects in insulin secretion and insulin action. A major feature of T2D is a lack of sensitivity to insulin by the cells of the body. Type 2 diabetes makes up about 90% of cases of diabetes and usually has its onset after age 40 years (David and Dolores, 2011). Riserus *et al*. (2009) mentioned that type 2 diabetes is due primarily to lifestyle factors and genetics, studies have shown that variants of the TCF7L2 gene increase susceptibility to type 2 diabetes for people who inherit two copies of the variants, the risk of developing type 2 diabetes is about 80 percent higher than for those who do not carry the gene variant. Also a number of lifestyle factors are known to be important to the development of type 2 diabetes, including obesity (defined by a body mass index of greater than thirty), lack of physical activity, poor diet and stress. On the other hand, rate of type 2diabetes have increased markedly since 1960 in parallel with obesity. As of 2010 there were approximately 285 million people diagnosed with the disease compared to around 30 million in 1985(Smyth and Heron, 2006). However, many individuals have symptoms of polyuria and polydipsia of several weeks duration. Polyphagia may be present, but is less common. Many people however, have no symptoms during the first few years and are diagnosed on routine testing. Ripsin *et al*. (2009) revealed that metformin is a first line treatment for type 2 diabetes, it works by decreasing production of glucose by the liver. Several other Groups of drug mostly given by mouth, may also decrease blood sugar in type 2 DM .

1.2.3.3 Gestational diabetes

It was defined as diabetes with onset or first recognition during pregnancy. Insulin resistance related to the metabolic changes of late pregnancy increase insulin requirements and may lead to impaired glucose tolerance. Raymond chang (2010) discussed that, if gestational diabetes untreated can cause damage to the health of the fetus or mother. On the other hand risk to the baby may include macrosomia (high birth weight), congenital cardiac and central nervous system anomalies, and skeletal muscle malformation. However, increased fetal insulin may cause respiratory distress syndrome. Also, a high blood bilirubin level may result if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia. Cooke and Plotnick (2008) found that, after pregnancy about 5_10% of women with gestational diabetes are found to have diabetes mellitus and most commonly type 2.

1.2.3.4 Other specific type

These type of diabetes may be due to genetic defect in B cell function, frequently characterized by mild onset of hyperglycemia at an early age (generally before 25). Formerly called Mody (maturity onset diabetes of the young), they have impaired insulin secretion with no defect in insulin action (Engelagau *et al*., 1995), and they may be due to genetic defect in insulin action (Green *et al.*, 1992; Bluestone *et al*., 2010). Gullo *et al*. (1994) noticed that they be due to disease of the exocrine pancreas such as pancreatitis and pancreatic carcinoma, endocrinopathies (pheochromocytoma, Cushing syndrome and acromegaly).

1.2.4 Complication

Studies have shown that diabetes doubles causes the risk of cardiovascular disease and about 75% of deaths in diabetics are due to coronary artery disease other macro-vascular disease are stroke and peripheral vascular disease (O'Gara *et al*., 2013). The primary complications of diabetes due to damage in small blood vessel

include :

1. Damage to the eyes known as diabetic retinopathy, is caused by damage to the blood vessel in retina of the eye, and can result in gradual vision loss and blindness (World Health Organization, 2014).

2. Damage to the kidney, known as diabetic nephropathy, can lead to tissue scarring urine protein loss, and eventually chronic kidney disease, dialysis or kidney transplant (World Health Organization, 2014).

3. Damage to the nerves of the body known as neuropathy, is the most common complication of diabetes (World Health Organization, 2014) .

1.2.5 Diabetes mellitus and thyroid disease

Diabetes mellitus and thyroid disease are the two common endocrinopathies seen in adults population (Johnson, 2006). The first reports showing the association between diabetes and thyroid dysfunction were published in 1979 (Feely and Isles, 1979; Gray and Irvine, 1979). Shah in 2007 found both insulin and thyroid hormones are involved in cellular metabolism and excess and deficit of either one can result in functional derangement of the other and thyroid disorders have similar symptoms to those of diabetes. Also, symptoms of hyperthyroidism may be attributed to poor diabetic control in patients with type 1 diabetes as such as despite increased appetite , fatigue and weight loss and these symptoms are common in patients with Type 2 diabetes. On the other hand, DM appears to influence thyroid function in two sites: firstly at the level of hypothalamic control of TSH release and secondly at the conversion of T4 to T3 in the peripheral tissue (Udoing *et al*., 2007).

1.2.6 Thyroid gland

Thyroid gland is a largest single endocrine gland. It is butterfly shaped gland with weight 20-25g, but it varies with age and physiological condition. It is found in the base of the neck just below the larynx. Thyroid gland produce important hormones tri-iodothyronine (T3) and tetra-iodothyroxine (T4). Thyroid hormones also regulate metabolism and organ function such as growth, brain development, fuel metabolism, body temperature and reproduction .On the other hand, thyroid hormones (T3 and T4) are produced by the follicular cells of the thyroid gland and regulated by TSH made by thyrotropes of the anterior pituitary gland . TSH production controlled by thyroid releasing hormone (TRH) produced by hypothalamus. That means hypothalamus and pituitary gland controls on thyroids hormonal secretion. Hypothalamus and pituitary gland decreaseTRH and TSH when T4 get to sufficient level in circulation (Goodman, 2003).

1.2.6.1 Synthesis of thyroid hormones

Iodide is absorbed from bloodstream by a process called iodide trapping, in this process, sodium is cotransported with iodide into cell. After iodide enters the cell is converted to iodine by enzyme called thyroid peroxidase. Also, iodine transfer into colloid by protein called pendrin, subsequently bound to tyrosine in a series steps to form monoiodotyrosine (MIT) and diiodotyrosine (DIT) as shown in figure (1-3). One molecule of MIT couples with DIT to form (T3) and two molecules of DIT combine to form (T4) (Satoru *et al.,* 2007). Irizarry and Lisandro(2014) reported that the major form of the thyroid hormone in the blood is Thyroxine (T4), which has a longer half_ life than T3. On the other hand Bernard (2007) mentioned that T4 converted to the active T3 with in cells by deiodinases.

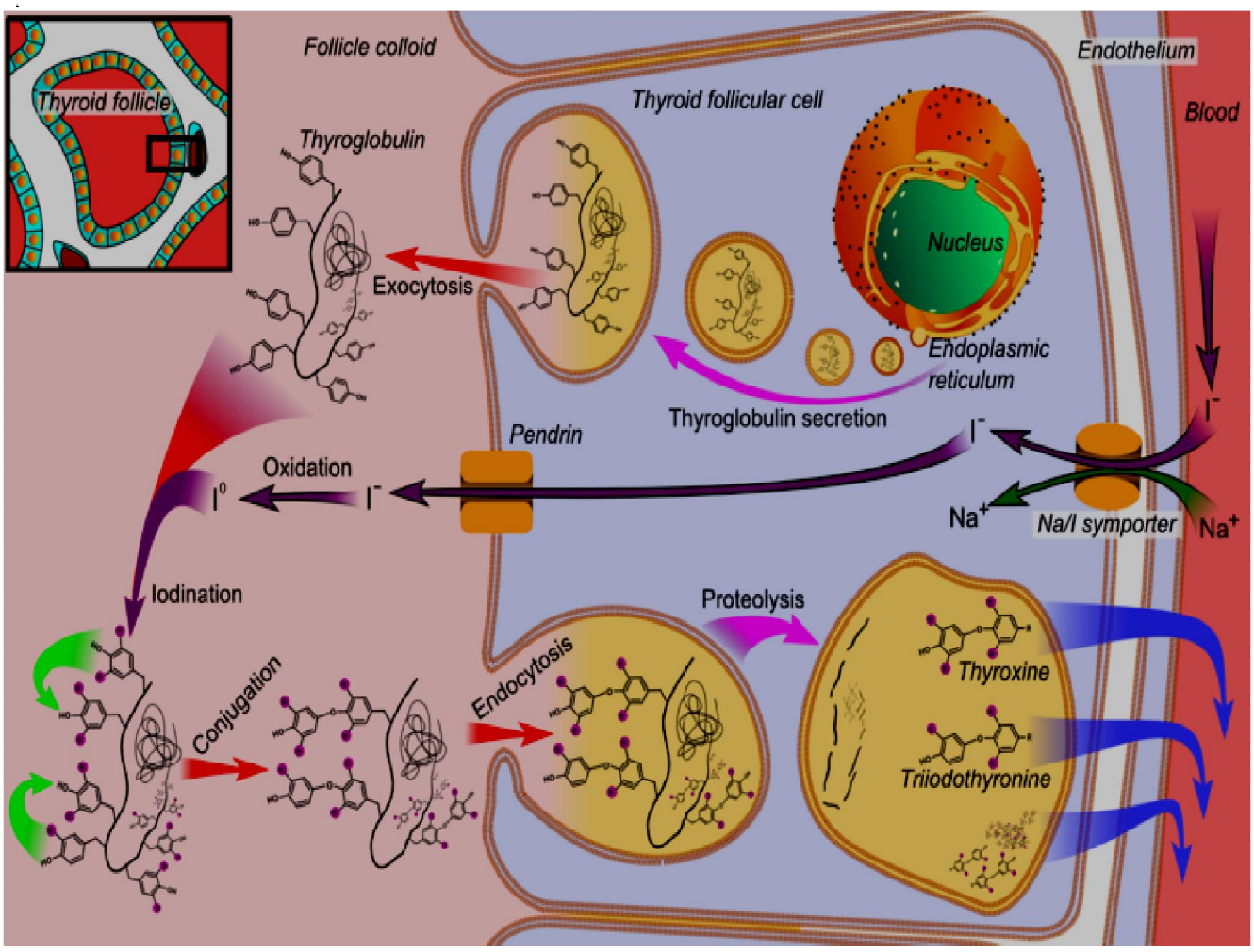

Figure (1-3) Steps in thyroid hormone synthesis (Boron and Boulpaep, 2003).

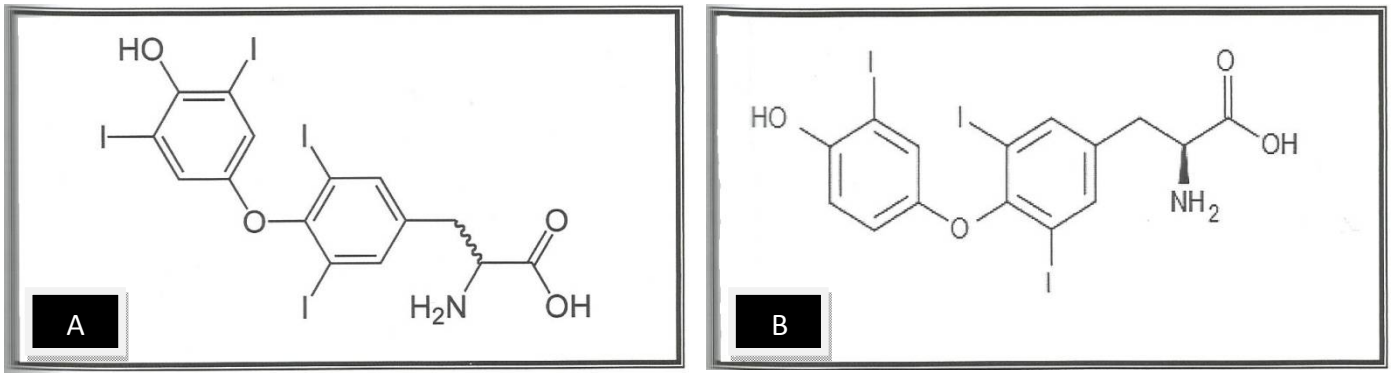

Figure (1-4) A : Thyroxin (T4) structure, B : Triiodothyronine (T3) structure (Boelaert and franklyn, 2005)

1.2.6.2 Transport of thyroid hormones

Albumin, thyroxin-binding globulin (TBG) and thyroxin binding prealbumin (TBPA) are three major thyroid hormones transport proteins. T3 and T4 have high affinity for TBG which allow it to carry about 70% of the circulating thyroid hormones. On the other hand, most of thyroid hormones (TH) in the plasma are coupled with proteins and less than 10% is free. These free hormones are in balance with the bound form (Eugster *et al*., 2004).

1.2.6.3 Thyroid disorders

Iodine is necessary for the production of T3 and T4. So a deficiency of iodine leads to decreased production of T3 and T4 and will cause the disease known hypothyroidism . While high amount of iodine cause increasing of synthesis rate of T3 and T4 and accumulation of T4 and T3 in the follicles, this is identified hyperthyroidism (Raven and Johnson, 1995; Richard, 2004).

A- Hypothyroidism

Hypothyroidism is a common metabolic disorder in the general population being the commonest thyroid disorder (Unnikrishnan and Menon, 2011).Also, it is occur when levels of thyroid hormones (tetra-iodothyroxine T4 and tri-iodothyroxine T3) are low and thyroid stimulating hormone (TSH) is high. On the other hand, it was found that hypothyroidism affects females about 2-8 times as often as males. While it can be referred to as an under-active thyroid gland caused in almost all cases by autoimmune disease (Brook and Marshal, 2001) . Thyroid hormones deficiency symptoms and signal include: thyroid gland enlargement, muscle pain, dry skin, weakness, hair loss, joint pain, reduce appetite, fingernails grow more slowly, become thicken, goiter and edema around eyes. It refer to heart rate slow down, circulation influence, intestinal activity decelerate. Other symptoms of hypothyroidism is the decreased ability to think some patients suffer loss of balance and difficulty in walking

(Zdraveska and Kocova, 2012). On the other hand, sub clinical hypothyroidism is characterized by normal serum concentration of tri-iodothyronine T3 and tetra-thyroxine T4 and increased serum concentration of thyroid stimulating hormone TSH (Greenspan and Gardner, 2004). It is common condition increased with age. Autoimmune thyroiditis (iodine deficiency, obesity and genetic abnormalities) considered important causes of subclinical hypothyroidism (Brown and Francis, 2011) .

B- Hyperthyroidism

In hyperthyroidism, a syndrome causes elevated levels of thyroid hormones (T3 and T4) and low level of thyroid stimulating hormone (TSH). This state is less common than hypothyroidism. However, the most common form of hyperthyroidism is the Graves's disease which is an autoimmune disease where auto antibodies bind to and activate thyroid stimulating hormone receptors that lead to stimulation of thyroid hormone synthesis (Ganong, 2003). Also, Brand and Gough (2011) mentioned that hyperthyroidism is caused toxic nodular goiter, in which appear one or more nodules appeared and become overactive then it will act as benign thyroid tumors. The major hyperthyroidism symptoms include: weight loss, tumor, nervousness, muscle weakness and sleep disturbances in addition to diarrhea and heart beat found by Iglesias *et al*.(2010).On the other hand, subclinical hyperthyroidism is defined as the arrangement of serum TSH suppressed concentration with normal serum free T3 and free T4 concentrations . Abnormal level of TSH may stay for years without clinical symptoms of clear hyperthyroidism. SCH can be caused by endogenous and exogenous factors . The endogenous form of subclinical hyperthyroidism (SCH) include thyroiditis , multinodular goiter, Grave's disease and other causes of hyperthyroidism. However, researchers associated subclinical hyperthyroidism with many the following risk factors such as: bone structure and metabolism, neuropsychiatric abnormalities and cardiovascular system (Diane, 2002; Biond, 2012).

1.2.6.3 Treatment of thyroid disease

Levothyroxine is the most commonly drug use for treatment of thyroid hormones deficiency (hypothyroidism), which is used synthetic thyroxine form and dose of this synthetic drug depending on body weight and age (Unnikrishnan and Jayakumar, 2010).

There are three methods for treatment of hyperthyroidism (Mahadevan , 2010) :

1. Medication: in which anti-thyroid drugs ,which is effect on the production and conversion of thyroid hormones.

2. Radioactive iodine: which iodine destroyed thyroid cells to reduce in size, thus reducing hormone levels.

3. Surgery: it is used to treatment thyroid nodules, and overactive thyroid , which is remove most of thyroid gland and become unable to producing hormones .

1.2.6.4 Autoimmune thyroid disease

Autoimmune thyroid disease (AITD) is characterized by the persistent activation of immunologic effecter mechanisms that alter the function and integrity of organ. This process of abnormal self-reactivity may be initiated by environmental and genetic agents. AITD affecting approximately 1.5% of the population, this is associated with female (He *et al*., 2007). It was found that others series conditions forms of autoimmune thyroid disease including hyperthyroid Graves' disease (GD), Hashimoto's (goiterous) thyroiditis, thyroid associated orbitopathy (TAO) , atrophic auto ammine hypothyroidism and postpartum thyroiditis (PPT). Graves ' disease(GD) and Hashimoto's thyroiditis(HT) are the commonest types one form of the disease may change to other as the course of the immune process (Deluca et *al*., 2013). On the other hand, the disorder of AITD share auto-antibodies to thyroid peroxidase (TPO),

thyroid stimulating hormone receptor (TSH_R) and thyroglobulin (TG) (Warren and Heymann, 2008). Graves' disease is characterized by thyroid hypersecretion and cellular hyperplasia caused by auto-antibodies (Laurbag *et al*., 2008) .Also auto-antibodies which is activate the thyroid stimulating hormone receptor (TSH_R), which is stimulates follicular hypertrophy and hyperplasia causing thyroid improve and increase synthesis and fraction of thyroid hormones (T3 and T4) . While Hashimoto's thyroiditis is common autoimmune disease caused by auto-antibodies that block the binding of TSH to its receptors without activating the receptors , result antibodies block the action of TSH and the extent of cellular infiltration and thyroid cell death. Patients with both disorders often have high levels of anti-thyroglobulin and anti-thyroid peroxidase antibodies (Brent, 2008).

1.2.6.5 Thyroid hormone effects on target tissues

Thyroid hormone regulates important functions in specific tissues such as:

1. Bone: TH is critical for normal bone growth and development. In children, hypothyroidism can cause short stature and delayed closure of the epiphyses. While biochemical studies have shown that TH can affect the expression of various bone markers in serum, reflecting changes in both bone formation and desorption (Allain and Mcgregor, 1993; Romani *et al.*, 1994). On the other hand TH increases osteocalcin in osteoblasts. TH may act on bone via TH stimulation of growth hormone or by direct effects on target genes (Mosekilde *et al*., 1990).

2. Heart: TH lowers systemic vascular resistance, increases blood volume, and has inotropic and chonotropic effects on cardiac function. The combination of these effect on both the circulation and the heart itself results in increased cardiac output. Hyper thyroid patients have a high output circulation state, whereas hypothyroid patients have low cardiac output, decreased stroke volume, decreased vascular volume and increased systemic vascular resistance. These changes in cardiac function by TH

ultimately depend on the regulation of target genes with the heart and indirect effect due to hemodynamic changes by TH (Klein, 1998).

3. Liver: TH has multiple effects on liver function including the stimulation of enzymes regulating lipogenesis and lipolysis as well as oxidative processes . Some of the lipogenic enzymes that are regulated malic enzyme, glucose-6-phoshpate dehydrogenase, and fatty acid synthase (Shekhar and Sinivas, 2011). It has been appreciated for many years that hypothyroidism is associated with hypercholesterolemia with elevated serum intermediate and low- density lipoprotein (LDL) cholesterol concentrations. The major mechanism for these effects may be lower cholesterol clearance resulting from decreased LDL (Tan *et al*., 1998).

4. Pituitary: TH regulates the synthesis and secretion of several pituitary hormones. TH also can negatively regulate thyrotropin (TSH) transcription by direct and indirect mechanisms. TH can negatively regulate thyrotropin releasing hormone (TRH) at the transcriptional level, which in turn decrease transcription of TSH mRNA (Samuels et al., 1988 ; Breen et *al.,* 1997).

5. Brain: TH has major effects on developing brain during the neonatal period (Bernal, 1999). Neonatal hypothyroidism due to genetic causes and iodine deficiency in humans can cause mental retardation and neurological defect (Rabie *et al.*, 1977) **.**

1.2.7 Diabetes mellitus and lipid metabolism

Patients with diabetes often present with abnormal lipid profile because insulin regulate several of the steps of lipid metabolism. Diabetic mellitus cause increase levels of low density lipoprotein cholesterol (LDL) , high Triglyceride (TG) and low of high density lipoprotein cholesterol (HDL), in addition DM lead to elevated of cholesterol level and this play role in increase risk of cardiovascular disease (CVD) (Ganong, 2003). On the other hand Jacobs *et al*. (2005) shows atherosclerosis to be found in patients with diabetes mellitus type 1 and change in lipid metabolism seems to play an important role in development of this complication. While diabetes mellitus type 2 is considered risk factor for the development of cardiovascular disease which lead to alteration in lipid profile (Arshag and Mooradian, 2009).

1.2.8 Thyroid and dyslipidemia

Thyroid hormones regulates the rate of both fat synthesis (lipogenesis) and lipolysis. T3 induces key lipogenic enzymes such as acetyl COA carboxylase and glucose-6-phosphate dehydrogenase and fatty acid synthase . The expression of these genes is also modulated by other factors such as high- carbohydrate diet, insulin and cAMP (Oppenheimer *et al.,*1991; Shekhar and Srinivas, 2011). Researchers demonstrated that the enzymes in the lipogenic pathway are regulated by thyroid hormone in both liver and adipose tissue . On the other hand, researchers make use of a new micro dialysis technique to study the effect of thyroidal state on lipolysis in vivo. They measured local release of norepinephrine (NE) and showed that (NE) concentrations at the adipocyte are greater in hyperthyroid patients and significantly less in hypothyroid patients compared with euthyroid controls (Haluzik *et al.*, 2003).

1.2.9 Lipid profile

1.2.9.1 Cholesterol

Cholesterol is a soft, waxy substance found in the blood stream and the body's cells, it is consist of four linked hydrocarbon rings forming the bulky steroid structure**.** There is a hydrocarbon tail linked to one end of the steroid and a hydroxyl group linked to the other end. Cholesterol is known as a "sterol" because it is made out of alcohol and steroid (Berg, 2002). It is carried by two types of lipoprotein; low-density lipoprotein (LDL) carried bad cholesterol and high-density lipoprotein (HDL) carried good cholesterol. Cholesterol is an extremely important biological molecule that has roles in contributes to the structure of cell walls, makes up digestive bile acids in the intestine, allows the body to produce vitamin D and enables the body to make certain hormones(Lewis and Rader, 2005).Synthesis of cholesterol, like that of most biological lipids, begins from the two-carbon acetate group of acetyl-CoA. The acetyl-CoA utilized for cholesterol biosynthesis is derived from an oxidation reaction (e.g., fatty acids or pyruvate) in the mitochondria and is transported to the cytoplasm by the same process as that described for fatty acid synthesis. Acetyl-CoA can also be synthesized from cytosolic acetate derived from cytoplasmic oxidation of ethanol which is initiated by cytoplasmic alcohol dehydrogenase (ADH). All the reduction reactions of cholesterol biosynthesis use NADPH as a cofactor.

The process of cholesterol synthesis can be considered to be composed of five major steps:

1. Acetyl-CoAs are converted to 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA)
2. HMG-CoA is converted to mevalonate
3. Mevalonate is converted to the isoprene based molecule, isopentenyl pyrophosphate (IPP) with the concomitant loss of CO_2
4. IPP is converted to squalene
5. Squalene is converted to cholesterol.

Hypercholesterolemia is a condition when there is an extremely high level of cholesterol in the body. Usually this means that there is a high concentration of LDL and low concentration of HDL. When too much LDL circulates the blood cell, it can built up the inner walls of arteries that feed the heart and brain, therefore, cause the clogging of the arteries. The health significance is that they are prone to cardiovascular diseases. If clot forms and blocks the narrowed artery, a series of cardiovascular diseases such as hypertension, arteriosclerosis, heart attack or stroke can result. High levels of cholesterol are also closely associated to diabetes (Lecerf and Lorgenl , 2011).

1.2.9.2 Triglycerides

A triglyceride is an ester derived from glycerol and three fatty acids. Triglycerides are the main constituent of body fat in humans and animals. There are also present in the blood to enable the bidirectional transference of adipose fat and blood glucose from the liver, and are a major component of human skin oils (Nelson, 2000). There are many different types of triglyceride, with the main division being between saturated and unsaturated types. Saturated fat are "saturated" with hydrogen all available places where hydrogen atoms could be bonded to carbon atoms are occupied. These hare a higher melting point and are more likely to be solid at room temperature. Unsaturated fats have double bonds between some of the carbon atoms, reducing the number of places where hydrogen atoms can bond to carbon atoms. These a lower melting point and are more likely to be liquid at room temperature (Alfred, 2002). The overall process of triglyceride biosynthesis consists of four biochemical pathways: fatty acyl-CoA biosynthesis, conversion of fatty acyl-CoA to phosphatidic acid, conversion of phosphatidic acid to diacylglycerol, finally conversion of diacylglycerol to triglycerol (Hemat, 2003).

1.2.9.3 High density lipoprotein (HDL)

High density lipoprotein is the smallest of the lipoprotein particales , it is composed of 80-100 proteins/particle which transport all fat molecule (lipids) around the body within the water outside cells. The fat carried include cholesterol, phospholipids, and triglycerides (Betteridge et *al*., 2008). The liver synthesizes lipoproteins as complex of apolipoproteins and phospholipid, which resemble cholesterol-free flattened spherical lipoprotein particles, the complexes are capable of picking up cholesterol carried internally from cells by interaction with the ATP-binding cassette transporter A1(ABC A1). A plasma enzyme called lecithin-cholesterol acyl transferase (LCAT) converts the free cholesterol into cholesterol ester (a more hydrophobic form of cholesterol), which is then sequestered into the core of the lipoprotein particle, eventually causing the newly synthesized HDL to assume a spherical shape. HDL particles increase in size as they circulate through the blood stream and incorporate more cholesterol and phospholipid molecules from cells and other lipoproteins (Huang and Zhang, 2013).

1.2.9.4 Low density lipoprotein (LDL)

Low density lipoprotein is one of the five major groups of lipoprotein, LDL has a highly hydrophobic core consisting of polyunsaturated fatty acid known as linoleate and hundreds to thousands esterified and unesterified cholesterol molecules. This core carries varying numbers of triglycerides and other fats and is surrounded by a shell of phospholipids and unesterified cholesterol (Segrest et *al.*, 2011). LDL particles are sometimes referred to as bad cholesterol because they can transport their content of fat molecules into artery walls, attract macrophages and thus atherosclerosis (Krauss, 2010).

Chapter two

Materials and Methods

Materials and methods

2.1 Materials

2.1.1 Apparatus and Equipments

The following apparatus and equipments were used in this study:

Apparatus	Company name	Origin
Centrifuge	kokusan	Japan
ELISA printer	Epson LX-300	Italy
ELISA Reader	Bioelisa reader EL800	Italy
ELISA Washer	Organon Teknlka	Australia
Freezer	Ishtar	Iraq
VIDAS	BioMereux	France
Spectrophotometer	Ce Cecil	Germany

2.1.2 Kits

Kits used in this study are listed as follows:

Kit	Company	Countries
Triiodothyronine (T3)	BioMeriuex	France
Thyroxin (T4)		
Thyroid stimulating hormone (TSH)		
C-peptide ELISA	Diametra	Italy
Cholesterol	Spainreact	Spain
Triglycerides		
HDL		

2 Methods

2.2.1 Subjects :

Ninety Iraqi diabetic patients were randomly selected from Al-Amal Hospital in Baghdad governorate during period from January 2015 to April 2015. Full information was taken from each patient about, type, history of diabetic family, risk factor information for body mass index (BMI) , any other disease , length and weight. Thirty apparently healthy volunteers were chosen as controls. Thyroid hormones , C-peptide, total cholesterol, triglyceride, low density lipoprotein and high density lipoprotein were measured in blood samples individual. The age of patients were range 20-60 years.

2.2.2 Blood samples

Five ml of venous blood samples were collected morning from each patient. Blood samples were centrifuged at 2000 r.p.m. for 10 minutes then serum of each sample was stored at $-20℃$.

2.2.3 Measurement of free triiodothyronine (FT3)

2.2.3.1 Principle

Enzyme linked fluorescent assay (ELFA) was used to determine FT3 in serum samples of diabetic patients and healthy control. Competition occurs between an unlabeled antigen (present in standard, control, and patient samples) and an enzyme-labeled antigen coated on the interior of the SPR (conjugate) for limited number of antibody binding sites on the micro plate. The washing and decanting procedures remove unbound materials. During the final detection step the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. Conjugate enzyme catalyzed the hydrolysis of this substrate into a fluorescent product measured at 450 nm . The intensity of the fluorescence is triiodothyronine present in the sample . At the end of

the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory , and then printed out.

2.2.3.2 Content of the kit (60TESTS)

- 60 FT3 strips (STR) .Ready to use**.**
- 60 FT3 SPRs 2× 30. Ready to use. SPRs sensitized with triiodothyronine**.**
- FT3 Control 1×2 ml (liquid)**.**
- FT3 Calibration 1×2 ml (liquid).
- 1 MLE card (Master Lot Entry) . Specifications for the factory master data required to calibrate the test.

2.2.3.3 Procedure

1. For each sample to be tested one "FT3" strip and one "FT3" SPR were used.

2. The test was identified by the "FT3" code on the instrument, the calibrator must be identified by "S1" and tested in triplicate. If the control is to be tested, it should be identified by "C1".

3. One hundred µl were mixed from calibrator, control and samples using a vortex type mixer (for serum or plasma separated from the pellet).

4."FT3" SPRs and "FT3" strips were inserted into the VIDAS(Vitek Immuno Diagnostic Assay System) instrument. All assay steps were done automatically by VIDAS instrument.

5. The assay was completed within approximately 40 minutes. After the assay is completed, the SPRs and strips were removed from the instrument.

6. Results of assay are analyzed and calculated automatically.

2.2.4 Measurement of free thyroxin (FT4)

2.2.4.1 Principle

Enzyme linked fluorescent assay (ELFA) was used to determine FT4 in serum samples of diabetic patients and healthy control . Competition occurs between an unlabeled antigen (present in standard, control, and patient samples) and an enzyme-labeled antigen coated on the interior of the SPR(solid phase receptacle) conjugate for

limited number of antibody binding sites on the micro plate. The washing and decanting procedures remove unbound materials. During the final detection step the substrate (4-Methyl-umbelliferyl phosphate) is cycled in and out of the SPR. Conjugate enzyme catalyzed the hydrolysis of this substrate into a fluorescent product measured at 450 nm . The intensity of the fluorescence is thyroxin present in the sample . At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory , and then printed out.

2.2.4.2 Content of the kit (60TEST)

- 60 FT4N Strips (STR). Ready to use.
- 60 FT4N SPRs 2×30 (SPR). Ready to use. Interior of SPR coated with thyroxine.
- FT4N Control 1×2 ml (liquid).
- FT4N Calibrator 1×2ml (liquid).
- 1 MLE Card (Master Lot Entry) Specifications for the factory master data required to calibrate the test**.**

2.2.4.3 Procedure

1. For each sample to be tested one "FT4N" strip and one "FT4N" SPR were used.

2. The test was identified by the "FT4N" code on the instrument, the calibrator must be identified by "S1" and tested in triplicate. If the control is to be tested, it should be identified by "C1".

3. One hundred µl were mixed from calibrator, control and samples using a vortex type mixer (for serum or plasma separated from the pellet).

4."FT4N" SPRs and "FT4N" strips were inserted into the VIDAS instrument. All assay steps were done automatically by VIDAS instrument.

5. The assay was completed within approximately 40 minutes. After the assay is completed, the SPRs and strips were removed from the instrument.

6. Results of assay are analyzed and calculated automatically.

2.2.5 Measurement of Thyroid Stimulating Hormone (TSH)

2.2.5.1 Principle

Thyroid stimulating hormone was measured by enzyme linked fluorescent assay. The sample is transferred into the well containing anti- TSH antibody labeled with alkaline phosphatase (conjugate). The sample/ conjugate mixture is cycled in and out of the SPR. The antigen binds to antibodies coated on the SPR and to the conjugate forming a "sandwich". Unbound components are eliminated during the washing steps. During the final detection step, the substrate (4-Methyl- umbelliferyl phosphate) is cycled in and out of the SPR. The conjugate enzyme catalyzes the hydrolysis of this substrate into a fluorescent product (4-Methyl- umbelliferone) the fluorescence of which is measured at 450nm. The intensity of fluorescence is proportional to the concentration of antigen present in the sample. At the end of the assay, results are automatically calculated by the instrument in relation to the calibration curve stored in memory, and then printed out.

2.2.5.2 Content of the kit (60 TESTS)

- 60 TSH strips (STR). Ready to use.
- 60 TSH SPRs 2×3 (SPR). Ready to use . SPRs sensitized with monoclonal anti TSH immunoglobulins (mouse).
- TSH control 1×3 ml (lyophilized).
- TSH calibrator 1×2 ml (lyophilized).
- TSH diluents 1×3ml (liquid).

2.2.5.3 Procedure

1. For each sample to be tested one "TSH" strip and one "TSH" SPR were used.

2. The test was identified by the "TSH" code on the instrument, the calibrator must be identified by "S1" and tested in triplicate. If the control is to be tested, it should be identified by "C1".

3. Two hundred µl were mixed from calibrator, control and samples using a vortex type mixer (for serum or plasma separated from the pellet).

4."TSH" SPRs and "TSH" strips were inserted into the VIDAS instrument. All assay steps were done automatically by VIDAS instrument.

5. The assay was completed within approximately 40 minutes. After the assay is completed, the SPRs and strips were removed from the instrument.

6. Results of assay are analyzed and calculated automatically.

2.2.6 Determination of total Cholesterol

2.2.6.1 Principle of the method

The cholesterol present in the sample originates a coloured complex, according to the following reaction:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholesterol} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-Aminophenazone} \xrightarrow{POD} \text{Quinonimine} + 4H_2O$$

The intensity of the red color formed is proportional to the cholesterol concentration in the sample.

2.2.6.2 Reagents

R1 buffer	Pipes pH6.9 Phenol
R2 enzymes	Cholesterol esterase (CHE) Cholesterol oxidase (CHOD) Peroxidase (POD) 4- Aminophenazone (4-AP)
Cholesterol CAL	Cholesterol aqueous primary standard

2.2.6.3 Procedure

1. The instrument was adjusted to zero with distilled water.

2. In to labelled test tubes the following reagent were pipetted.

	Blank	Standard	
WR	1.0ml	1.0ml	1.0ml
Standard	_ _ _	10 μL	_ _ _
Sample	_ _ _	_ _ _	10 μL

3. The tubes were mixed, then incubated for 10 min at 25℃ .

4. The absorbance (A) of the samples and standard were measured at 505nm using the following equation:

Total cholesterol = $\frac{(A)\text{Sample}}{(A)\text{Standard}} \times$ concentration of Standard(200mg/dL).

2.2.7 Determination of Triglycerides

2.2.7.1 Principle of the method

Triglyceride sample was incubated with lipoprotein lipase (LPL) to liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). In the last reaction, hydrogen peroxide (H_2O_2) reacts with 4- aminophenazone (4-AP) and p-chlorophenol in presence of peroxidase (POD) to give a red colored dye as shown in the following equations:

$$\text{Triglycerides} + \text{H2O} \xrightarrow{LPL} \text{Glycerol} + \text{free fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{GK} \text{G3P} + \text{ADP}$$

$$\text{G3P} + \text{O2} \xrightarrow{GPO} \text{DAP} + \text{H2O2}$$

$$H_2O_2 + \text{4-AP} + \text{p-Chlorophenol} \xrightarrow{POD} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the triglycerides concentration in the sample.

2.2.7.2 Reagents

R1 buffer	Good pH7.5 p-Chlorophenol
R2 Enzymes	Lipoprotein lipase (LPL) Glycerolkinase (GK) Glycerol-3-oxidase (GOP) Peroxidase(POD) 4-Aminophenazone (4-AP) ATP
Triglycerides CAL	Triglycerides aqueous primary standard

2.2.7.3 Procedure

1. The instrument was adjusted to zero with distilled water.

2. In to labeled test tubes the following reagent were pipetted.

	Blank	Standard	Sample
WR	1.0 ml	1.0 ml	1.0 ml
Standard	_ _ _	10 μL	_ _ _
Sample	_ _ _	_ _ _	10 μL

3. Tubes were mixed, then incubated for 10 min. at 25°C.

4.The absorbance (A) of the samples and standard were measured at 505nm using the following equation:

$$\text{Triglycerides} = \frac{(A)\text{Sample}}{(A)\text{Standard}} \times \text{concentration of Standard(200mg/dL)}$$

2.2.8 Determination HDL

2.2.8.1 Principle of the method

The very low density lipoproteins (VLDL) and low density lipoproteins (LDL) from serum or plasma are precipitated by phosphotungstate in the presence of magnesium ions. After centrifugation the supernatant contains high density lipoproteins (HDL) . The HDL fraction is determined using the total cholesterol enzymatic reagent.

2.2.8.2 Procedure:

1. Aliquots of 100μl of the reagent were mixed with 1ml of serum sample in labeled test tubes.

Reagent	100 µL
Sample	1.0 mL

2. Tubes were incubated for 10 min 25℃

3. Test tubes were centrifuged at 4000 r.p.m. for 20 min or 2 min at 12000 r.p.m.

4. Supernatant was collected carefully, then aliquots of 50μl of supernatant were added to 1ml of cholesterol reagent.

5. The tubes were mixed and incubated for 10 min at room temperature.

6. Absorbance (A) of samples and standard were measured at 505nm using the following equation:

$$\text{HDL} = \frac{(\text{A})\text{Sample}}{(\text{A})\text{Standard}} \times \text{concentration of Standard(200mg/dL)}$$

LDL = Total cholesterol− HDL – (TG/5) mg/dl.

VLDL = Triglyceride /5(mg/dl)

2. 2.9 Body Mass Index (BMI) calculation

Body Mass Index (B.M.I) is a simple index of weight for height that is commonly used to classify under weight, over weight and obesity in adults . It is defined as the weight in kilograms divided by the square of the height in meters (kg/m^2).

2.2.10 Determination of C-peptide

2.2.10.1 Principle

The C-peptide ELISA kit is solid phase enzyme- linked immune sorbent (ELISA), based on the principle of competitive binding. The microplate are coated with anti-bodies, which bind a monoclonal antibody directed towards a unique antigenic sit on C-peptide molecule. Endogenous C-peptide of a patient sample competes with a C-peptide- horseradish peroxidase conjugate for binding to the coated antibody. After incubation the unbound conjugate is washed off. The amount of bound peroxidase conjugate is inversely proportional to the concentration of C-peptide in the sample. After addition of the substrate solution, the intensity of color developed is inversely proportional to the concentration of C-peptide in the patient sample.

2. 2.10.2 Reagents

1. C-peptide calibrators: 6 vials, 2ml each,

2.Conjugate: (1 vial, 13 ml) antibodies anti C-peptide conjugated with horseradish peroxidase (HRP) and anti C-Peptide biotinilated.

3.TMB Substrate : (1 vial, 15 ml) H_2O_2. TMB 0.26 g/L

4.Stop Solution: (1vial, 15 ml) Sulphuric acid 0.15 mol/L

5. Wash Solution: (1 vial, 20 ml) NaCL

2.2.10.3 Procedure.

1. The labeled tubes were pipetted according to the following volumes:

Reagent	Calibrator	Sample	Blank
C_0-C_5 calibrator	50µL		
Sample		50µL	
Conjugate	100µL	100µL	

2. Microplate was incubated at 25℃ for 2 hour then washed 3times with 300µL of diluted Wash Solution.

3. One hundred µl of TMB substrate were added to each sample ,calibrator and blank and the microplate was incubated at room temperature for 15 minutes in the dark. After that 100 µl of stop solution were added to each sample, calibrator and blank.

4. The microplate gently was shacked and the absorbance (A) was measured at 450 nm.

5. The mean of the absorbance was calculated for each point of the calibration and samples.

6. Finally, the curve was draw to determine the concentration of C-peptide for unknown then, the intersecting point was found on the curve and the concentration was read from horizontal axis graph.

2.2.11 Statistical Analysis:

The following statistical data analysis approaches were used in order to analyze and assess the results of the study under application of the statistical package for social sciences (SPSS) version 10 :

1. **Descriptive data analysis**:

a- Tables (Frequencies, and Percentages).

b- Mean value, Standard Deviation, Standard Error, (95%) Confidence interval for population mean parameter, and two extremes values (min. and max.) for assuming that data under lying followed (Normal Distribution Function).

c- Simple Person's correlation coefficients: A measure of linear correlation between two quantitative variables. Values of the correlation coefficient range from -1 to 1. The sign of the coefficient indicates the direction of the relationship, and its absolute value indicates the strength, with larger absolute values indicating stronger relationships.

d- Contingency Coefficients for the association tables: A measure of association between two qualitative variables. Values of the correlation coefficient range from 0 to 1.

e- Graphical presentation by using :

- Bar Chart.
- Cluster Bar Chart.

2. Inferential data analysis:

These were used to accept or reject the statistical hypotheses, which included the following :

a- The One-Way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Analysis of variance is used to test the hypothesis that several means are equal, as well as (Least Significant Difference (LSD), and Games Howell (GH)) tests are accounted after rejecting the statistical hypotheses, for equal or unequal variances are assumed respectively.

b- Simple Person's correlation coefficient test.

c- Contingency Coefficients (C.C.) test for the cause's correlation ship of the association tables.

$$C.C. = \sqrt{\frac{\chi^2}{\chi^2 + T..}}$$

Where χ^2 is the Chi Square statistic and T.. is the overall total of the contingency table.

For the abbreviations of the comparison significant (C.S.), we used the followings:

- NS : Non significant at P>0.05
- S : Significant at P<0.05
- HS : Highly significant at P<0.01

Chapter Three

Results and Discussion

3. Results and discussion

3.1 Subjects

A total of ninety subjects were included in this study. Thirty of them were healthy controls, while other thirty were diabetic patients suffering from hypothyroidism and another thirty patients suffering from hyperthyroidism. Almost patients were also suffering from high blood pressure and heart disease (atherosclerosis and palpitations). Blood samples were collected from those patients to study the relationship between hyper and hypothyroidism cases with type 2diabeties disease by detecting blood tests and other physical and sociological parameters.

3.2 Distribution of diabetic patients and control groups according to gender

Gender of type 2 diabetic patients with hypo and hyperthyroidism was taken in regards to study the relationship between gender and the infection with those diseases. Results in table (3-1) and

figure (3-1) showed that, diabetic males with hypothyroidism are 8, 33.3%, and diabetic males with hyperthyroidism are 9, 37.5% in comparison with 7, 29.2% males control. On the other hand, 21, 31.8% diabetic females are hyperthyroidism, 22, 33.3% diabetic females are hypothyroidism in comparison with 23, 34.8% female controls.

According to gender of patients and healthy groups, statistical analysis showed that there is no significant differences between hypothyroidism compared to control and hyperthyroidism with control. This result is agreed with Mussa *et al.* (2005) and Erdogan *et al.,* (2010) who they mentioned that there is no significant difference between studied groups according to gender.

Table (3-1): Comparative between studied group according to gender

Parameters	Classes	No. & Percent	Groups			C.S. (*) P-value
			Control	Hypo.	Hyper.	
Gender	Male	No.	7	8	9	C.C.=0.061 P=0.843 NS
		% Gender	29.2%	33.3%	37.5%	
	Female	No.	23	22	21	
		% Gender	34.8%	33.3%	31.8%	

(*) NS: Non Sig. at P>0.05 ; C.C. : Contingency Coefficient (Association)

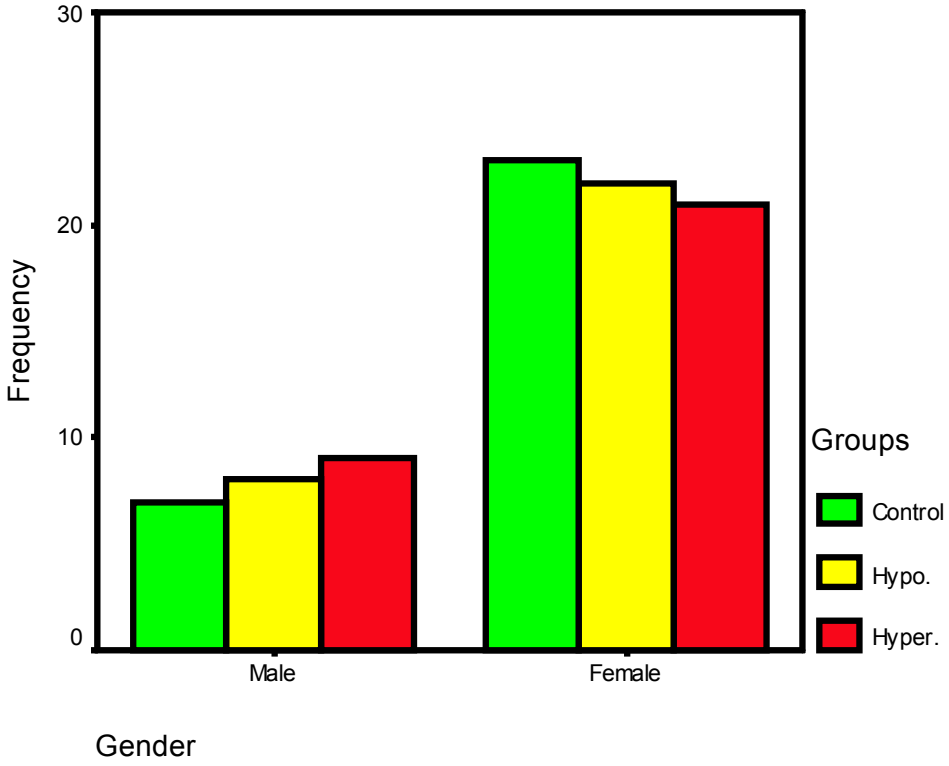

Figure (3-1): Distribution of diabetic patients according to gender

Figure (3-1) showed that there is significant different between female and male in study groups this due to stress ,fatigue in addition to pregnancy affects on the immunity of women and causing thyroid disorders this study agreed with another studies by Vander (2013) and Darwish *et al.,* (2006) thyroid disorders were found to be higher in females than in males with ratios of 10:1 and 3:1

,respectively . Mahadevan (2010) stated that thyroid disorders are more prevalent in females (with an incidence of roughly 8 to 10 times) more than in males.

3.3 Distribution of patients and control groups according to age

Results indicated in table (3-2) and figure (3-2) showed that the mean age of diabetic patients with hypothyroidism and hyperthyroidism was 45.1 and 42.8 years respectively compared to 38.4 years for controls with no significant difference. This result indicated that there is no significant difference with hypo and hyperthyroidism and age of adults patients compared to healthy controls. These results are agreed with other results obtained in other studies (El-Hefnawi *et al.,* 2004; Fraranak *et al.*, 2007) which they mentioned that there is no relationship in age between hypo and hyperthyroidism diabetic patients and control groups.

Table (3-2): Distribution of study groups according to age

Age Groups	No. & Percent	Groups			C.S. (*) P-value
		Control	Hypo.	Hyper.	
20 - 29	No.	2	0	0	P=0.173 NS
	% Age Groups	100%	0.0%	0.0%	
30 - 39	No.	14	7	9	
	% Age Groups	46.7%	23.3%	30.0%	
40 - 49	No.	11	17	16	
	% Age Groups	25.0%	38.6%	36.4%	
50 - 59	No.	3	6	5	
	% Age Groups	21.4%	42.9%	35.7%	
Mean ± SD		38.4 ± 8.1	45.1 ± 6.3	42.8 ± 7.1	

(*) NS: Non Sig. at P>0.05 ;

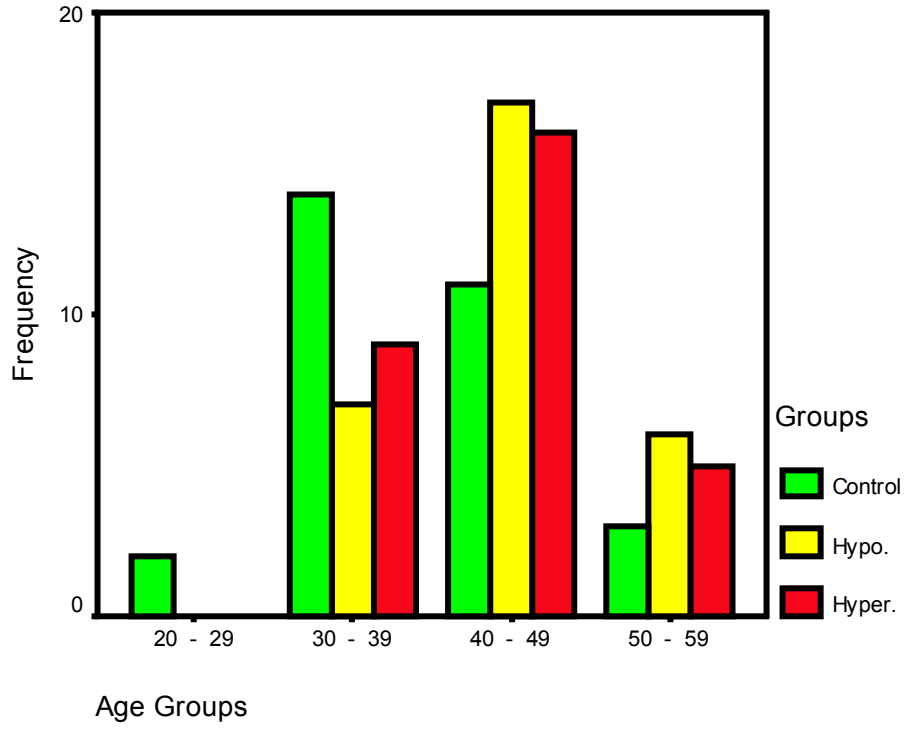

Figure (3-2): Distribution of diabetic patients according to age

3.4 Distribution of diabetic patients and control groups according to body mass index (BMI)

Body mass index is another indicator biomarker for diabetes in relation with hypo and hyperthyroidism, BMI is classified to normal weight (18.50 – 24 kg /m^2) over weight (25 – 30kg / m^2) and obese (>30 kg/ m^2).

Results in table (3-3) showed that the average of body mass index for normal weight in healthy controls, diabetic patients with hypothyroidism and diabetic patients with hyperthyroidism are 41.7, 25.0, 33.3 respectively, while in overweight are 51.4, 20.0, 28.6 on the other hand, the average of body mass index in healthy controls, diabetic with hypothyroidism and diabetic with hyperthyroidism for obese are 16.3, 46.5, 37.2 respectively with significant difference between each group. These results

showed that the body mass index was affected by the incidence of diabetes associated with hypothyroidism or hyperthyroidism and age of patients with diabetic affected on body mass index (Erdogan et *al.*, 2010). On the other hand, Koritschon (2011) mentioned that there is significant differences in BMI between diabetic patients with hypothyroidism, hyperthyroidism and control.

Table (3-3): Distribution of body mass index (BMI)between studied groups

Parameters	Classes	No. & Percent	Groups			Total	C.S. P-value
			Control	Hypo.	Hyper.		
BMI	Normal weight	No.	5	3	4	12	C.C.=0.346 P=0.016 S
		% BMI	41.7%	25.0%	33.3%	100%	
	Overweight	No.	18	7	10	35	
		% BMI	51.4%	20.0%	28.6%	100%	
	Obese	No.	7	20	16	43	
		% BMI	16.3%	46.5%	37.2%	100%	

S: Sig. at P>0.05; Testing hypotheses are bases on : Contingency Coefficient (C.C.) of association test, as well as Binomial test.

3.5 Relationship between diabetes and thyroid dysfunctions

Results in table (3-4) and figure (3-3) showed that FT3 was highly significant different at (P<0.01) between hypothyroidism and control groups, as well as significant different at (P<0.05) between hypothyroidism and hyperthyroidism groups, and finally no significant different between hyperthyroidism and control groups.

While FT4 results shows no significant different at P>0.05 are accounted among studied groups.

Finally, TSH results showed that there is highly significant difference between hypothyroidism and hyperthyroidism groups (at $P<0.01$), while no significant different at $P>0.05$ are accounted between hyperthyroidism and control groups.

The abnormal thyroid hormone levels in diabetic patients may be due to medications. It is known that insulin is an anabolic hormone which enhances the level of T4 while suppress the level of T3 by inhibiting hepatic conversion of T4 to T3. This result was agreed with other studies achieved by Suzuki et *al*., 1994; Radaieh *et al.*, 2004 and Afkhami *et al.*, 2010 who mentioned that altered thyroid hormone level of different magnitude (both low and high) in diabetic patients. Also Priti *et al*.(2014) found that high level of TSH and FT3 in diabetic patients type 2 compared with non diabetics, from 100 diabetic cases studied 29% showed abnormal thyroid hormone levels (24% hypothyroidism and 5% hyperthyroidism. On the other hand, Venkateshwarlu *et al*. (2012) mentioned that forty patients with DM type 2 showed abnormal thyroid function in a study sample of 200 compared with control.

Table (3-4) Statistics analysis summary of Thyroid Parameters (FT3, FT4, and TSH) in the studied groups

Parameter	Group	No.	Mean	Std. Dev.	Std. Error	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
FT3	Control	30	5.79	1.26	0.23	5.32	6.27	3.94	8.52
	Hypo.	30	4.48	1.14	0.21	4.05	4.90	1.00	6.25
	Hyper.	30	5.35	2.08	0.38	4.57	6.13	3.21	11.36
FT4	Control	30	15.07	2.62	0.48	14.10	16.05	10.51	19.18
	Hypo.	30	15.08	3.71	0.68	13.70	16.47	1.24	19.81
	Hyper.	30	14.94	4.46	0.81	13.28	16.61	7.75	25.96
TSH	Control	30	1.88	1.02	0.19	1.50	2.26	0.35	5.00
	Hypo.	30	7.21	12.31	2.25	2.62	11.81	0.07	60.00
	Hyper.	30	0.96	0.97	0.18	0.60	1.33	0.05	3.37

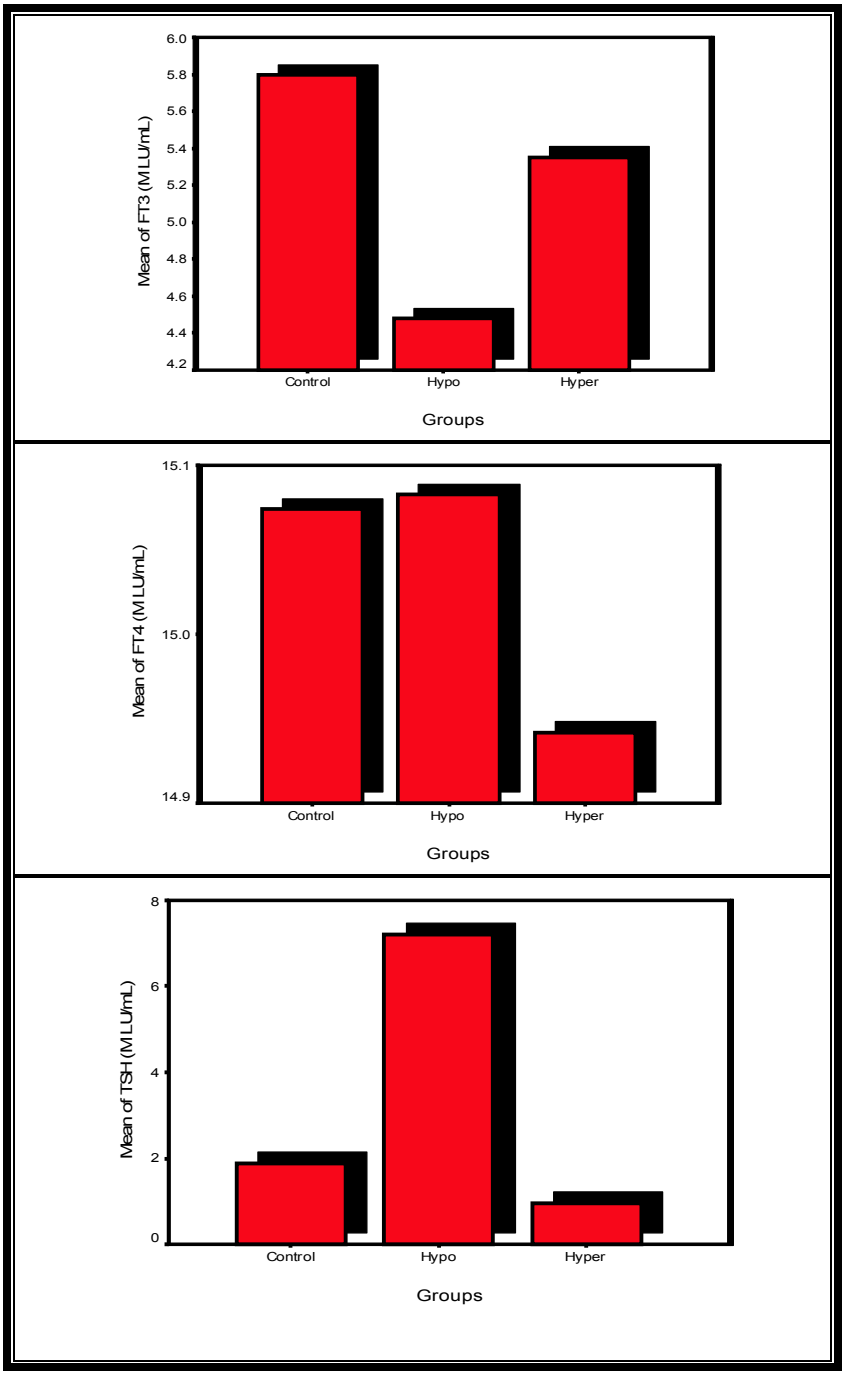

Figure (3-3): Mean values for "FT3, FT4, TSH" parameters in the studied groups

Figure (3-3) showed that FT3 and TSH parameters could be used as a good indicator for studying diabetic patients, while FT4 parameter according to mean, and variance values couldn't be reliable for discriminate diabetic disease, rather than some patients are accounted border to lower and upper critical normal values of

hypothyroidism and hyperthyroidism groups respectively compared with control group this due to drugs .

Table (3-5): Testing equality of Means and equality of variances for Thyroid Function parameters among the studied groups

Parameters	Levene's Test for Equality of Variances		ANOVA for Equality of Means		C.S. (*)
	L-test	Sig.	F-test	Sig. (2-tailed)	
FT3	1.671	0.194	5.613	0.005	HS
FT4	1.772	0.176	0.014	0.986	NS
TSH	1.812	0.170	13.014	0.000	HS

(*) HS: Highly Sign. at P< 0.01; NS: Non Sign

Table (3-5) showed ANOVA technique results for testing equality of mean values concerning "Thyroid Functions" parameter's readings among different of studied groups, as well as Levene test for testing equality of variances are calculated. Results shows that a highly significant differences are accounted for FT3, and FT4 parameters in light of testing equality of mean values at $P<0.01$, while FT4 parameter recorded no significant different at $P>0.05$ among studied groups. and for testing equality of means at $P<0.01$. In addition to that, equality of variances tests, shows that assumption equality variances of three independent groups are true, since no significant are accounted at $P<0.05$.

Table (3-6): Multiple Comparison using (LSD) method for Thyroid hormones

Parameters	(I) Group	(J) Group	Sig.	C.S. (*)
TF3	Control	Hypo.	0.001	HS
		Hyper.	0.270	NS
	Hypo.	Hyper.	0.032	S
TF4	Control	Hypo.	0.993	NS
		Hyper.	0.890	NS
	Hypo.	Hyper.	0.882	NS
TSH	Control	Hypo.	0.000	HS
		Hyper.	0.135	NS
	Hypo.	Hyper.	0.001	HS

(*) HS: Highly Sig. at P< 0.01; S: Sig. at P> 0.05 ; NS: Non Sig. at P> 0.05

3.6 Lipid profile comparison between diabetic patients and control groups

Thyroid diseases are associated with various metabolic abnormalities due to the effect of thyroid hormones on the major metabolic pathways (Peppa *et al.,*2011). The explanation of thyroid hormones affected on lipid metabolism is that thyroid hormone regulates the activity of some key enzymes in lipoproteins transport and ,therefore, alter the lipoprotein levels in hypothyroid patients (Saini *et al,* 2012).

The result indicated table (3-7) and figure (3-4) showed that the level of total cholesterol as recorded in control, hypothyroidism and hyperthyroidism groups mean values 168.8, 225.6,168.0 mg/dl respectively, while triglyceride level showed that mean values were recorded in control 113.1 mg/dl, hypothyroidism 169.9 mg/dl and hyperthyroidism 88.2 mg/dl. As well as, low density lipoprotein (LDL) level results in the mean values of 97.9 mg/dl for control group, 119.8 mg/dl for hypothyroidism, while hyperthyroidism showed 90.5 mg/dl. On the other hand, very low density lipoprotein (VLDL) level results were recorded the mean values 27.1 mg/dl in control, hypothyroidism with 33.6 mg/dl and hyperthyroidism 17.7 mg/dl.

Finally, high density lipoprotein (HDL) level result declared that mean values were recorded in control, hypo and hyperthyroidism 48.8, 69.5, 80.1 mg/dl.

According to the lipid profile in control and patients groups statistical analysis showed that total cholesterol result in high significant differences at ($P<0.01$) between hypothyroidism and control groups. Also, significant differences at ($P<0.05$) between hypo and hyperthyroidism groups where as no significant different at ($P>0.05$) between hyperthyroidism and control groups. Triglyceride results also showed that there is a significance differences at ($P<0.05$) between studied groups. On the other hand, LDL results recorded that no significant differences at $P>0.05$ between the studied groups. Where as a high significant differences at $P<0.01$ between hypothyroidism and hyperthyroidism were recorded in VLDL test and no significant differences at $P>0.05$ between studied groups. Finally, high significant differences at $P<0.01$in HDL were obtained between hypothyroidism and control groups from one side and between hyperthyroidism and control groups from other side, while no significant differences at $P>0.05$ between hypo and hyperthyroidism.

The result in this study is recorded the agreement with the study of Muhammad *et al*., 2009 who found that total cholesterol, triglyceride and HDL were significant increased in diabetic patients compared to control also, this study was closely related with Sawant *et al* .,2008 who they found the abnormally high concentration of serum lipid in diabetes compared to control. On the other hand, Raghad et al., 2014 reported that significant differences $P<0.05$ when compared lipid in diabetic patients with thyroid disorders and non diabetics.

Table (3-7): Statistical analysis summary of the "Lipid Profiles" test in the studied groups

Parameters	Group	No.	Mean	Std. Dev.	Std. Error	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
Total Cholesterol	Control	30	168.8	32.6	6.0	156.7	181.0	102	216
	Hypo.	30	225.6	58.4	10.7	203.8	247.4	129	432
	Hyper.	30	186.0	37.3	6.8	172.1	200.0	113	259
Triglyceride	Control	30	113.1	38.7	7.1	98.6	127.5	51	187
	Hypo.	30	169.9	107.5	19.6	129.8	210.1	56	578
	Hyper.	30	88.2	35.6	6.5	74.9	101.5	55	214
LDL	Control	30	97.9	31.9	5.8	86.0	109.8	36	161
	Hypo.	30	119.8	62.6	11.4	96.5	143.2	23	347
	Hyper.	30	90.5	37.4	6.8	76.6	104.5	28	173
VLDL	Control	30	27.1	26.5	4.8	17.2	37.0	10	161
	Hypo.	30	33.6	21.5	3.9	25.6	41.6	11	115
	Hyper.	30	17.7	7.0	1.3	15.1	20.3	11	42
HDL	Control	30	48.8	8.4	1.5	45.7	51.9	35	65
	Hypo.	30	69.5	31.9	5.8	57.6	81.4	17	124
	Hyper.	30	80.1	33.1	6.0	67.8	92.5	23	146

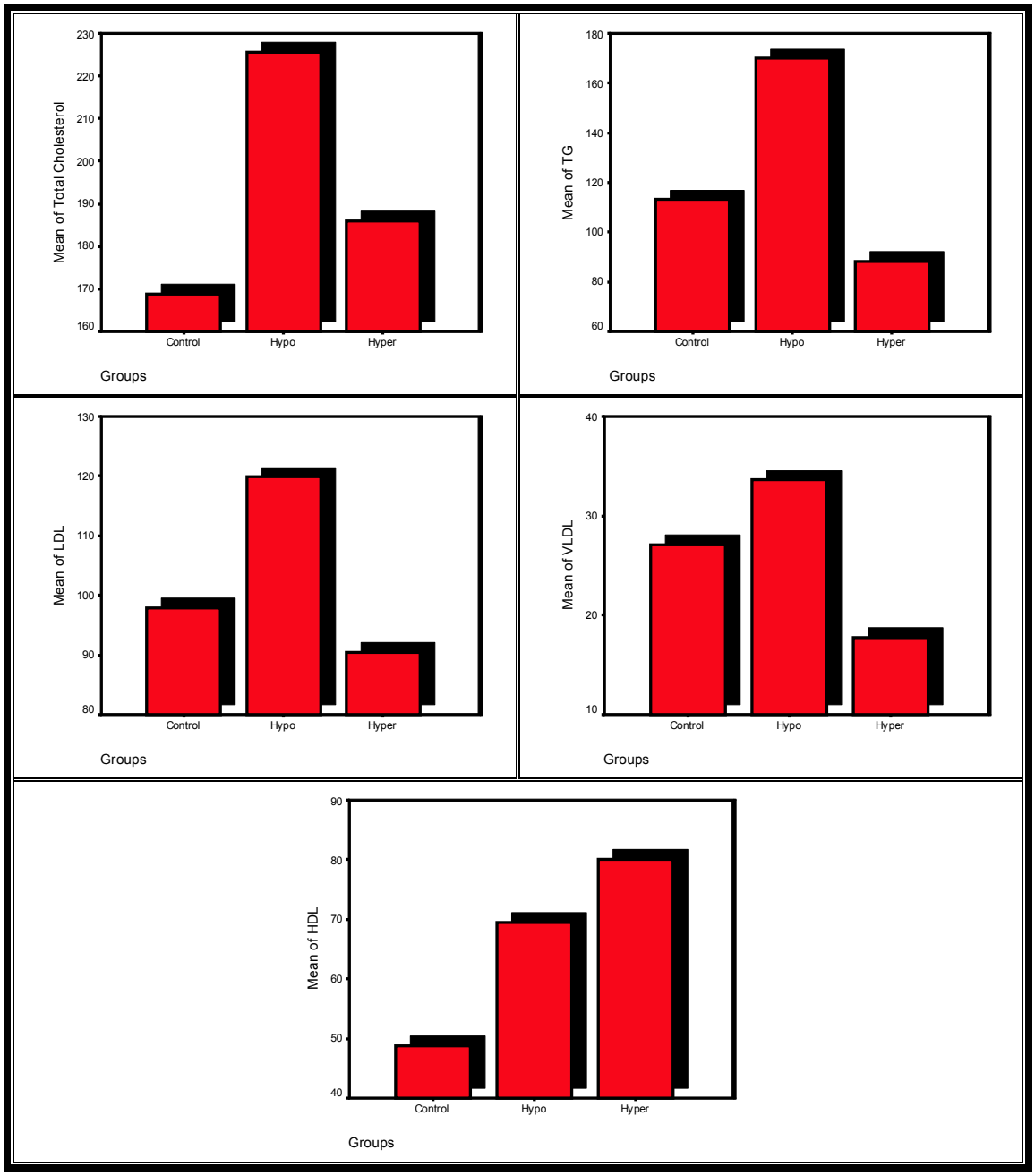

Figure (3-4): Mean values of " Cholesterol, Triglyceride, LDL, VLDL, and HDL" in the studied groups

Figure (3-4) showed that High level of cholesterol ,triglyceride and HDL in diabetic patients with hypo and hyperthyroidism this result because insulin resistance lead to increased metabolic of lipid to release energy compensation for decrease of glucose in body and that lead to increased HDL.

Table (3-8): Testing equality of Means and equality of variances for Lipid Profiles parameters among the studied groups

Parameters	Levene's Test for Equality of Variances		ANOVA for Equality of Means		C.S. (*)
	L-test	Sig.	F-test	Sig. (2-tailed)	
Cholesterol	1.81	0.170	13.014	0.000	HS
Triglyceride	11.2	0.000	0.236	0.814	NS
LDL	3.30	0.043	-2.130	0.041	S
VLDL	3.40	0.036	-0.369	0.713	NS
HDL	20.8	0.000	-2.141	0.040	S

(*) HS: Highly Sig. at P< 0.01; S: Sig. at P> 0.05 ; NS: Non Sig. at P> 0.05

Table (3-8) showed a highly significant different at P<0.01 had been registered in testing equality of means with respect of Cholesterol, as well as significant differences at P<0.05 are accounted in light of LDL, and HDL, while no significant differences among studied groups at P>0.05 with respect of Triglyceride, and VLDL parameters, as well as equality of variances test, shows that assumption of three variances are equality at each parameter are not true, since significant results are accounted in at least at P<0.05 in light of studied parameters of lipid profiles, except cholesterol parameter, since had reported no significant level at P>0.05.

Table (3-9): Multiple Comparison using (LSD) and (GH) methods for all pair of contrasts

Parameters	(I) Group	(J) Group	Sig.	C.S. (*)
Total Cholesterol	Control	Hypo.	0.000	HS
		Hyper.	0.135	NS
	Hypo.	Hyper.	0.001	HS
TG	Control	Hypo.	0.026	S
		Hyper.	0.032	S
	Hypo.	Hyper.	0.001	HS
LDL	Control	Hypo.	0.212	NS
		Hyper.	0.691	NS
	Hypo.	Hyper.	0.081	NS
VLDL	Control	Hypo.	0.553	NS
		Hyper.	0.162	NS
	Hypo.	Hyper.	0.001	HS
HDL	Control	Hypo.	0.004	HS
		Hyper.	0.000	HS
	Hypo.	Hyper.	0.419	NS

(*) HS: Highly Sig. at P< 0.01; S: Sig. at P> 0.05 ; NS: Non Sig. at P> 0.05

3.7 Relationship between C-peptide and thyroid disease in the studied groups

The result in table (3-10) and figure (3-5) showed that the mean values of control are 1.2 ng/dl while mean values of hypothyroidism are 2.9 ng/dl compared 3.1 ng/dl for hyperthyroidism group.

According to results of C-peptide for patients and control statistical analysis showed that C-peptide test gave high significant different at ($P<0.01$) between hypothyroidism and control groups and between hyperthyroidism and control groups, while there is no significant differences were recorded between hypo and hyperthyroidism.

In a similar study, Radaideh *et al.,* 2004 and Afkhami (2010) who found that both insulin and thyroid hormones are involved in cellular metabolism and excess and deficit of any one can result in functional derangement of other.

Table (3-10): Statistics analysis summary of [C- Peptide] Parameter in studied groups

Parameter	Group	No.	Mean	Std. Dev.	Std. Error	95% C.I. for Mean		Min.	Max.
						L.B.	U.B.		
C. peptide	Control	30	1.2	0.3	0.1	1.1	1.3	0.7	1.8
	Hypo.	30	2.9	1.5	0.3	2.3	3.4	0.7	6.6
	Hyper.	30	3.1	1.5	0.3	2.5	3.6	1.1	6.9

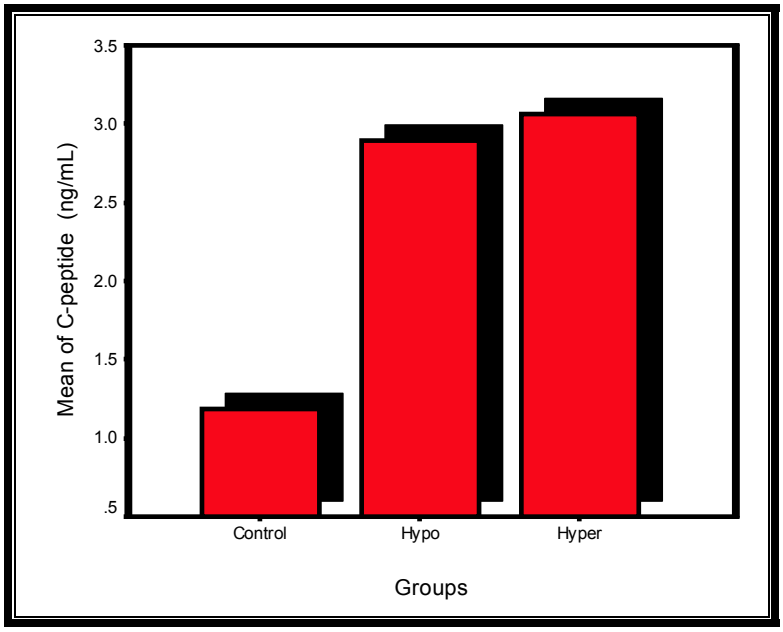

Figure (3-5): Mean values for "C- Peptide" parameter in the studied groups

Figure (3-5) showed that high level of C-peptide in patients with hypo and hyperthyroidism this because thyroid hormones directly control insulin secretion , in hypothyroidism there is a reduction in glucose induced insulin secretion by beta cells, and the response of beta cells to glucose is increased in hyperthyroidism due

to increased beta cell mass and cell do not response to insulin so that due to increase C- peptide.

Table (3-11):Testing equality of Means and equality of variances for C-Peptide parameter among the studied groups

Parameter	Levene's Test for Equality of Variances		ANOVA for Equality of Means		C.S. (*)
	L-test	Sig.	F-test	Sig. (2-tailed)	
C-peptide	18.890	0.000	21.665	0.000	HS

(*) HS: Highly Sig. at P< 0.01

Table (3-11) showed ANOVA technique results for testing equality of mean values concerning "C- Peptide" parameter's readings among different of studied groups, as well as Levene test for testing equality of variances are calculated. Results shows that a highly significant different are accounted for "C- Peptide" parameter in light of either for testing equality of mean values or for testing equality of variances at P<0.01, and with respect to that continuing comparisons significant throughout using (GH) method, which are reposed on equal variances are not assumed, and that illustrated in.

Table (3-12): Multiple Comparison using (GH) method for testing equality of means for all pairs of contrasts

Parameters	(I) Group	(J) Group	Sig.	C.S. (*)
C. peptide	Control	Hypo.	0.000	HS
		Hyper.	0.000	HS
	Hypo.	Hyper.	0.896	NS

(*) HS: Highly Sig. at P< 0.01; NS: Non Sig. at P> 0.05

Chapter four

Conclusions and Recommendations

4.1 Conclusions

1. High incidence of abnormal thyroid hormones level were observed in type 2 diabetic patients.
2. There were significant correlation with high level of FT3 , TSH and total cholesterol and triglyceride in diabetic patients with thyroid disorders.
3. There were significant difference in age and body mass index .
4. gender in type 2 diabetic patients is not a significantly differ from that observed in healthy control.
5. There is significant difference in C- peptide between groups.
6. There is significant correlation between triglyceride and very low density lipoprotein and high density lipoprotein.

4.2 Recommendations

1. Screening thyroid hormones level for all diabetic patients.

2. Further studies should be taken to establish the dietary pattern of type 2 patients in Iraq and other factors that may lead to dyslipidemia in those patients.

3. Further study on adiponectin protein in hypo and hyperthyroidism patient adults and correlation with diabetes mellitus.

4. Genetic study of glucose transporting protein (GLUT-2) in liver of thyroid disease patients .

5. Study of thyroid peroxidase (TPO) as marker for thyroid patients.

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Appendix

Appendix I: Case profile

Name	
Sex	
Age	
Weight	
Length	
Other diseases	
C- peptide	
Lipid profile	
CH	
TG	
HDL	
LDL	
VLDL	
Thyroid hormones	
FT3	
FT4	
TSH	
Hyperthyroidism ( )	**Hypothyroidism ( )**

Appendix II : Result of Control

No. of Sample	FT3	FT4	TSH	Total Cholesterol	TG	LDL	VLDL	HDL	C-peptide	Sex	Length Cm	Weight K gm	Age	BMI
1	5.18	16.41	1.64	216	131	145	26	45	0.8	f	158	76	32	3.05
2	5.92	12.64	1.59	167	187	90	37	40	0.9	f	160	75	35	29.2
3	4.40	12.72	0.86	216	161	143	32	41	0.9	f	158	82	33	32.9
4	4.80	18.99	5.00	205	170	106	34	65	1.2	f	156	77	38	31.6
5	5.33	15.77	2.32	118	97	38	19	61	0.9	f	160	78	34	30.4
6	3.94	18.54	2.32	108	51	54	10	44	0.7	m	158	68	54	27.3
7	5.83	14.67	2.87	178	119	105	23	50	1.1	f	175	90	30	29.4
8	6.48	11.69	0.90	102	72	53	14	35	1.5	f	163	87	41	32.8
9	4.12	19.18	1.57	145	59	92	11	42	1.1	f	165	85	31	31.3
10	4.31	11.21	0.39	145	136	81	27	37	1.8	m	175	70	30	22.8
11	4.32	11.13	1.13	183	93	120	18	45	1.6	f	160	65	32	25.3
12	7.12	18.11	2.03	145	170	51	34	60	0.8	f	156	63	29	25.9
13	5.11	12.41	2.12	205	80	131	161	58	1.3	m	175	79	55	25.8
14	6.12	13.25	1.60	167	97	86	19	62	1.3	m	164	63	25	23.5
15	5.41	14.11	4.30	189	114	119	22	48	1.8	m	179	73	32	22.8
16	7.12	13.31	2.34	216	68	161	13	42	1.1	f	162	70	46	26.7
17	8.52	17.42	1.18	200	102	141	20	39	1.5	m	164	69	56	25.7
18	6.74	16.15	2.17	168	97	94	19	55	1.4	f	158	60	45	24
19	4.13	15.93	0.57	199	148	127	29	43	0.8	f	159	61	35	24.2
20	4.63	10.51	0.35	200	140	123	28	49	1.2	f	165	75	47	20.8
21	7.33	17.13	1.64	167	120	78	24	65	1.1	f	160	78	40	30.4
22	6.34	18.12	1.41	178	131	95	26	56	1.8	m	163	74	41	27.9
23	8.1	12.57	2.98	180	160	96	32	52	1.2	f	167	70	43	25.17
24	**5.11**	**13.56**	**2.45**	**175**	**83**	**112**	**16**	**50**	**0.8**	**f**	**166**	**76**	**37**	**27.6**
25	**5.42**	**13.79**	**1.02**	**160**	**75**	**97**	**15**	**48**	**0.7**	**f**	**159**	**69**	**40**	**28.3**
26	**6.72**	**15.31**	**1.76**	**177**	**83**	**110**	**16**	**51**	**0.9**	**f**	**160**	**75**	**45**	**29.2**
27	**4.71**	**14.46**	**1.66**	**150**	**59**	**100**	**11**	**39**	**1.7**	**f**	**165**	**80**	**45**	**29.4**
28	**6.72**	**16.61**	**2.67**	**156**	**180**	**75**	**36**	**46**	**1.1**	**f**	**169**	**79**	**32**	**27.7**
29	**7.51**	**18.55**	**1.92**	**145**	**112**	**77**	**22**	**46**	**1.3**	**f**	**160**	**67**	**40**	**26.1**
30	**6.35**	**17.97**	**1.73**	**105**	**97**	**36**	**19**	**50**	**1.4**	**f**	**158**	**74**	**30**	**30.4**

Appendix III : Result of Hyperthyroidism

No. of Sample	FT3	FT4	TSH	Total Cholesterol	TG	LDL	VLDL	HDL	C-peptide	Sex	Length Cm	Weight K gm	Age	BMI
1	4.14	15.27	1.71	178	98	63	19	96	1.4	f	162	98	36	37.4
2	5.64	14.45	1.13	189	64	111	13	65	1.5	f	160	79	46	30.8
3	4.13	14.35	0.05	162	102	62	16	84	3.6	f	171	108	37	36.9
4	5.24	12.58	0.05	216	55	84	11	121	1.3	f	161	70	40	27
5	5.36	15.13	0.05	189	59	147	11	31	1.8	f	150	81	47	36
6	4.63	10.53	0.94	237	59	80	11	146	1.1	f	150	84	30	37.3
7	7.44	19.63	0.05	173	59	63	11	99	2.7	f	162	96	45	36.6
8	4.27	15.88	0.39	237	76	104	15	118	4.2	f	158	68	45	27.3
9	4.55	9.97	3.32	113	55	28	14	71	2.3	f	162	72	34	27.4
10	4.23	11.02	0.65	210	174	67	34	109	6.9	m	175	69	50	23.3
11	4.36	12.71	0.05	243	102	102	20	121	5.0	f	158	80	35	32.1
12	4.05	9.85	0.05	221	72	95	14	112	3.2	f	155	83	32	35.4
13	11.36	25.96	0.05	156	55	56	16	84	4.0	m	169	94	54	0.35
14	4.65	16.02	3.37	156	76	73	15	68	2.1	f	155	87	31	36.2
15	3.75	7.75	0.05	145	80	48	16	81	2.5	m	170	82	54	28.3
16	4.88	11.13	2.68	194	89	68	17	109	4.0	f	165	69	32	25.3
17	3.21	9.06	0.27	147	72	127	14	118	1.7	f	160	79	32	30.8
18	11.21	20.0	0.05	259	80	65	16	66	6.6	m	170	70	45	24.2
19	10.53	25.13	0.05	130	73	42	26	62	4.0	m	175	82	48	27.7
20	4.55	14.26	0.05	162	103	70	20	72	4.2	m	165	68	50	25
21	4.98	19.84	1.3	135	96	78	19	37	2.1	f	150	56	42	0.01
22	4.94	13.34	1.6	145	148	72	29	43	2.1	m	165	86	46	31.6
23	4.84	16.19	1.0	160	103	98	20	41	3.7	m	160	77	47	30.6
24	**4.72**	**9.47**	**1.3**	**225**	**214**	**159**	**42**	**23**	**4.6**	**f**	**158**	**84**	**47**	**33.7**
25	**5.26**	**13.22**	**1.5**	**216**	**55**	**84**	**11**	**121**	**2.1**	**f**	**159**	**79**	**45**	**31.3**
26	**6.07**	**19.17**	**1.4**	**180**	**93**	**173**	**18**	**42**	**2.5**	**m**	**170**	**89**	**45**	**34.2**
27	**4.86**	**19.77**	**1.7**	**219**	**73**	**153**	**14**	**50**	**4.1**	**f**	**155**	**65**	**47**	**27**
28	**4.60**	**16.55**	**1.1**	**195**	**89**	**68**	**17**	**109**	**2.4**	**f**	**160**	**76**	**50**	**29.6**
29	**3.72**	**14.84**	**1.3**	**204**	**89**	**131**	**17**	**55**	**2.1**	**f**	**169**	**77**	**47**	**27**
30	**4.34**	**15.19**	**1.7**	**185**	**83**	**144**	**16**	**50**	**2.1**	**f**	**154**	**63**	**46**	**26.5**

Appendix IV: Result of Hypothyroidism

No. of Sample	FT3	FT4	TSH	Total Cholesterol	TG	LDL	VLDL	HDL	C-peptide	Sex	Length Cm	Weight K gm	Age	BMI
1	3.78	14.61	29.78	221	250	100	50	71	6.6	m	165	106	45	38.9
2	1	1.24	60.0	140	233	95	46	115	2.3	m	160	100	49	39
3	2.77	11.87	11.83	237	114	116	22	99	2.5	f	158	94	35	37.7
4	3.10	16.71	20.97	205	72	89	14	102	4.2	m	173	104	47	34.7
5	3.34	13.17	11.86	313	161	210	32	71	1.2	f	160	92	37	35.5
6	3.64	12.93	17.71	189	72	110	14	65	3.2	f	155	67	38	27.9
7	3.73	6.41	15.18	167	114	58	22	87	0.7	f	158	50	36	20
8	3.92	17.37	6.47	243	170	126	34	84	2.9	f	155	83	48	34.5
9	4.23	16.42	9.05	221	89	95	17	109	3.1	f	158	78	42	31.3
10	4.54	16.28	1.16	237	123	120	24	93	1.8	f	156	65	44	26.6
11	5.88	17.26	0.84	129	114	33	22	74	1.5	m	158	100	54	40.1
12	6.19	19.81	0.07	162	114	81	22	59	5.3	f	160	88	37	34.3
13	3.31	12.31	1.36	205	191	89	38	78	5.2	f	160	71	47	27.7
14	5.17	12.98	3.69	210	140	104	28	78	1.6	f	156	72	45	29.6
15	4.72	13.63	3.69	243	114	97	22	124	1.7	m	158	72	45	28.9
16	4.39	15.92	0.31	237	578	23	115	99	2.0	f	160	113	34	44.1
17	4.32	17.00	0.69	291	123	177	24	90	1.9	f	158	80	43	22.1
18	5.00	17.91	3.98	297	93	205	18	74	3.3	f	160	94	39	36.7
19	4.19	15.14	1.89	237	153	86	30	121	2.3	f	160	63	40	25.9
20	5.29	15.60	0.91	432	85	347	17	68	1.2	f	158	75	46	30.1
21	4.74	18.20	1.9	240	148	94	29	26	1.2	f	150	100	55	44
22	4.68	17.30	1.1	225	300	145	60	20	3.6	f	158	93	48	37.3
23	5.46	15.61	1.5	180	311	68	62	49	3.1	f	156	70	49	28.8
24	**4.48**	**17.33**	**1.3**	**195**	**318**	**69**	**63**	**21**	**2.1**	**m**	**158**	**90**	**47**	**36.1**
25	**5.22**	**16.78**	**1.1**	**158**	**56**	**88**	**11**	**57**	**2.3**	**m**	**175**	**70**	**53**	**23.3**
26	**6.25**	**18.24**	**1.3**	**203**	**126**	**143**	**25**	**34**	**4.7**	**f**	**150**	**99**	**43**	**44**
27	**4.36**	**17.13**	**0.9**	**225**	**300**	**145**	**60**	**20**	**3.5**	**m**	**169**	**99**	**56**	**34.7**
28	**4.79**	**14.35**	**3.3**	**260**	**200**	**144**	**40**	**17**	**2.1**	**f**	**162**	**95**	**52**	**36.2**
29	**6.25**	**18.24**	**1.3**	**203**	**126**	**143**	**25**	**34**	**4.7**	**f**	**154**	**69**	**44**	**29.1**
30	**5.58**	**14.73**	**1.2**	**263**	**110**	**195**	**22**	**46**	**5.0**	**f**	**162**	**85**	**55**	**32.4**

Appendix V: blood glucose in control and diabetic patients

1	92 mg/dl
2	69 mg/dl
3	72 mg/dl
4	75 mg/dl
5	63 mg/dl
6	88 mg/dl
7	81 mg/dl
8	86 mg/dl
9	77 mg/dl
10	71 mg/dl
11	82 mg/dl
12	95 mg/dl
13	87 mg/dl
14	85 mg/dl
15	70 mg/dl
16	85 mg/dl
17	89 mg/dl
18	88 mg/dl
19	107 mg/dl
20	71 mg/dl
21	112 mg/dl
22	73 mg/dl
23	96 mg/dl
24	77 mg/dl
25	81 mg/dl
26	90 mg/dl
27	107 mg/dl

28	87 mg/dl
29	69 mg/dl
30	90 mg/dl
31	144 mg/dl
32	192 mg/dl
33	215 mg/dl
34	126 mg/dl
35	109 mg/dl
36	250 mg/dl
37	188 mg/dl
38	200 mg/dl
39	225 mg/dl
40	203 mg/dl
41	242 mg/dl
42	148 mg/dl
43	264 mg/dl
44	307 mg/dl
45	264 mg/dl
46	121 mg/dl
47	300 mg/dl
48	150/ mg/dl
49	217 mg/dl
50	192 mg/dl
51	163 mg/dl
52	240 mg/dl
53	124 mg/dl
54	307 mg/dl
55	109 mg/dl

56	371 mg/dl
57	120 mg/dl
58	196 mg/dl
59	244 mg/dl
60	121 mg/dl
61	254 mg/dl
62	237 mg/dl
63	206 mg/dl
64	175 mg/dl
65	252 mg/dl
66	238 mg/dl
67	166 mg/dl
68	175 mg/dl
69	360 mg/dl
70	184 mg/dl
71	331 mg/dl
72	259 mg/dl
73	213 mg/dl
74	270 mg/dl
75	222 mg/dl
76	180 mg/dl
77	207 mg/dl
78	112 mg/dl
79	200 mg/dl
80	178 mg/dl
81	303 mg/dl
82	180 mg/dl
83	263 mg/dl

84	379 mg/dl
85	196 mg/dl
86	193 mg/dl
87	303 mg/dl
88	139 mg/dl
89	153 mg/dl
90	252 mg/dl

الخلاصة

ركزت الدراسة على مسح الاعراض الغير طبيعية للغده الدرقية (ارتفاع وانخفاض مستوى هرمونات الغده الدرقية) وتقدير مستوى هذه الهرمونات (هرمون الغده الدرقية الثلاثي الحر ،هرمون الغده الدرقية الرباعي الحر والهرمون المحفز للغده الدرقية) بين مرضى عراقيين مصابين بداء السكري من النوع الثاني. كما تم مقارنة مستوى الدهون (الكوليسترول الكلي ، الكليسريدات الثلاثية، الدهون البروتينيه عالية الكثافة ، الدهون البروتينيه المنخفضة الكثافة و الدهون البروتينيه المنخفضة الكثافة جدا) لمرضى السكري من النوع الثاني مع مجموعة الاصحاء الغير مصابين بهذا المرض .

شملت الحالات في هذه الدراسه تسعون مصابا بمرض السكري من النوع الثاني ثلاثون مصابا يعانون من ارتفاع مستوى هرمونات الغدة الدرقية وثلاثون مصاباً اخر يعانون من انخفاض مستوى هرمونات الغده الدرقية. وقد اختيرت ثلاثون عينة دم من افراد اصحاء غير مصابين بمرض السكري وامراض الغده الدرقية كمجموعة تحكم.

بينت النتائج ارتفاع مستوى هرمونات الغده الدرقية بشكل غير طبيعي عند الاشخاص المصابين بمرض السكر من النوع الثاني حيث كان مستوى هرمون الغده الدرقية الثلاثي الحر لمرضى السكري الذين يعانون من ارتفاع مستوى هرمونات الغدة الدرقية (5.35)µL/mL في حين الاشخاص الذين يعانون من انخفاض مستوى هرمونات الغدة الدرقية كان µL/mL (4.48) مقارنة بالاصحاء µL/mL (5.79).وكان مستوى هرمون الغده الدرقية الرباعي الحر لمرضى السكري الذين يعانون من ارتفاع وانخفاض مستوى هرمونات الغده الدرقية على التوالي كان µL/mL (14.94,15.08) مقارنة بالاصحاء (15.07)µL/mL. بينما كان مستوى الهرمون المحفز للغده الدرقية لمرضى السكري الذين يعانون من ارتفاع وانخفاض مستوى هرمونات الغده الدرقيه على التوالي كان µL/mL (0.96,7.21) مقارنة بالاصحاء (1.88)µL/mL.

كذلك اظهرت نتائج التحليل الاحصائي وجود علاقه ايجابيه $P<0.05$ بين ارتفاع الكوليسترول والدهون الثلاثيه عند الاشخاص المصابين بارتفاع وانخفاض مستوى هرمونات الغده الدرقية .

كما اثبتت النتائج وجود علاقة معنوية بين معدل العمر ودليل كتلة الجسم عند الاشخاص المصابين بمرض السكري من النوع الثاني مقارنة بالاصحاء.

كذلك اظهرت نتائج التحليل الاحصائي اصابة النساء باضطرابات الغدة الدرقية اكثر من الرجال.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

دراسة المقارنه بين هرمونات الغده الدرقية والدهون عند المصابين بالسكر النوع الثاني

رسالة
مقدمة الى مجلس كلية العلوم/جامعة النهرين
كجزء من متطلبات نيل درجة الماجستير علوم/ تقانة احيائية

من قبل
نور اسعد عبود المطوري
بكالوريوس علوم الحياة/كلية العلوم /جامعة البصره
(2012)

اشراف
د.اسماء علي حسين
(استاذ مساعد)

شوال
1437هـ

تموز
2016 م