Libyan International Medical University
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Association between Thyroid Dysfunction and Type II Diabetes Mellitus among Adults in Benghazi City

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List of contents

List of tables

List of figures

Abbreviations

2-h PG: two-hour plasma glucose

HbA1C: glycated hemoglobin

Anti-TG: anti-thyroglobulin antibody

Anti-TPO: anti-thyroid peroxidase antibody

BMI: body mass index

CVD: cardiovascular disease

DAG: diacylglycerol

DR: diabetic retinopathy

ER: endoplasmic reticulum

FFA: free fatty acids

FPG: fasting plasma glucose

GLUT-2: glucose transporter-2

HHEX: hematopoietically expressed homeobox

HHS: hyperglycemic hyperosmolar state

Ig: immunoglobulin

IGF2BP2: insulin-like growth factor 2, binding protein 2

IKK: IκB kinase complex

IL: interleukin

IRS: insulin receptor substrate

KCNJ11: potassium inwardly rectifying channel, subfamily J, member 11

NF-κB: nuclear Factor kappa-light-chain-enhancer of activated B cells

NPDR: non-proliferative diabetic retinopathy

OGTT: oral glucose tolerance test

PKC: protein kinase C

PDR: proliferative diabetic retinopathy

T1DM: type 1 diabetes mellitus

T2DM: type 2 diabetes mellitus

T3: triiodothyronine

T4: thyroxine

TCF7L2: transcription factor 7-like 2

TLR-4: toll like receptor-4

TNF: tumor necrosis factor

TRH: thyrotropin releasing hormone

TSH: thyroid-stimulating hormone

Abstract

Diabetes mellitus is one of the most common endocrine disorders and is often classified as type 1 and type 2 according to its pathogenicity. Type 2 is due insulin resistance, which is when the target cells don't respond to insulin. It is seen more in older age groups and associated with obesity. Insulin and thyroid hormones influence one another, if the thyroid function is disturbed in diabetic patients; the glycemic control is negatively affected. The aim of the study was to assess the frequency and pattern of thyroid dysfunction in type 2 diabetic patients. The participants (n=90) included diabetic patients (n=71) and control (n=19). Thyroid status was evaluated and 8.45% of diabetic patients were shown to have subclinical hypothyroidism, while in the control group, the prevalence of subclinical hypothyroidism was 10.53%. No other types of thyroid disorders were found. Statistical analysis was performed and showed no significant relationship between thyroid dysfunction and the following: gender, age, BMI, duration of diabetes, usage of insulin and oral hypoglycemic agents or hypertension, in either diabetic or control group. The prevalence of thyroid autoimmunity among the patients was 14.08%. The prevalence was higher in females than males (20.51% vs. 6.25%) in the diabetic group as well as in the control group (16.67% vs. 14.29%).

1. Introduction

1.1. Diabetes Mellitus

Diabetes mellitus is a chronic metabolic disease characterized by hyperglycemia. Normally when levels of glucose are elevated in the blood, the insulin is released from pancreatic cells and acts to increase the cells' uptake of glucose; where it's used to release energy (muscle cells) or stored for later use (liver and fat cells). In diabetes, it is caused by either a defect in insulin secretion or an insufficient response from the target cells (Dean and McEntyre, 2004).

Chronic hyperglycemia causes a long-term and possibly life-threatening complications which include retinopathy, nephropathy and neuropathy (Association, 2014). It also causes an increased risk of heart disease ranging from to 2-to-4 folds. Diabetic patients are classified according to the pathogenicity of the disease. Most are classified into two types: either there is a complete absent of insulin due to autoimmune destruction of pancreatic beta cells, referred to as type 1 diabetes mellitus (T1DM); or there is a resistance to insulin action, referred to as type 2 diabetes mellitus (T2DM). It is common for insulin resistance to be accompanied by defects in its secretion (Olokoba, Obateru and Olokoba, 2012).

Symptoms of hyperglycemia include polyuria, polydipsia and polyphagia. If left untreated, uncontrolled diabetes could lead to ketoacidosis or non-ketotic hyperosmolar syndrome. The early symptoms are mild and hyperglycemia could go untreated for years; especially in T2DM (Olokoba, Obateru and Olokoba, 2012).

Diabetes mellitus is considered one of the most common endocrine disorders worldwide. The region of the Middle East and North Africa has a prevalence of 10.9% (Kharroubi, 2015). T1DM occurs mostly among children under 15 years with a higher incidence in people of European descent. T2DM has a much higher prevalence, and its incidence increases with age. However, it is becoming more common in children due to increased rate of obesity (Forouhi and Wareham, 2019).

1.1.1. Epidemiology

The incidence of T1DM varies among regions, the highest being in Europe where Sardinia and Finland have an incidence rate of 37.8 and 40.9 per 100,000 per year respectively (Karvonen, 2006). The incidence is high to intermediate in North America, intermediate in Africa and lower in Asia. The peak incidence is during puberty, with an increase in the male population. Worldwide, there is an increase of 3% per year; mostly in youngest age groups (Forouhi and Wareham, 2019).

Regarding T2DM, it is seen more among certain ethnicities. Studies in the United Kingdom have shown that its prevalence increases in South Asians and African-Caribbeans (Gholap *et al.*, 2011; Tillin *et al.*, 2013). The prevalence was estimated to be 425 million in 2017 and expected to reach 629 million in 2045. There is a slight difference in the prevalence between genders, with an estimated 17 million more men than women (Forouhi and Wareham, 2019).

Among the Arab countries; Saudi Arabia, Kuwait and Qatar are among those with the highest prevalence worldwide. In Saudi Arabia, the prevalence had increased from 2.4-4.3% thirty years ago to 25% in recent years (Al-Rubeaan *et al.*, 2015). A survey in Kuwait found T2DM in 48% of obese males and 77% of obese females (Alarouj *et al.*, 2013). Other studies in Arab countries also showed association with obesity; in Oman (60.1%) (Al-Moosa *et al.*, 2006), and Qatar (59.7%) (Bener *et al.*, 2009).

Children are becoming more susceptible to T2DM. Between 2001 and 2009; the prevalence had an increase of 30.5% among children and young adults in the United States. This is most likely due to the increased prevalence of obesity among younger age groups (Dabelea *et al.*, 2014).

1.1.2. Risk factors

While researchers haven't found a specific cause for the development of diabetes; it has been associated with several risk factors. Genetics is strongly believed to play a role; seeing how the rates are higher among monozygotic twins. It was also found that 40% of T2DM first-degree relatives are at risk of developing diabetes (Wu *et al.*, 2014).

Nearly 75 susceptibility loci have been linked to T2DM in genome-wide association studies. These loci include: potassium inwardly rectifying channel, subfamily J, member 11 (KCNJ11) which encodes ATP-sensitive potassium channel; transcription factor 7-like 2 (TCF7L2) which controls the regulation of pro-glucagon gene expression and the production of glucagon-like peptide 1; Insulin receptor substrate 1 (IRS-1) which affects the insulin action; insulin-like growth factor 2 binding protein 2 (IGF2BP2) which is involved in development of pancreas and stimulation of insulin action, and hematopoietically expressed homeobox (HHEX) which has an effect on beta cell development (Wu *et al.*, 2014).

Lifestyle factors have a big impact in developing diabetes. Obesity poses a large risk. Compared with individuals of normal weight, the risk of developing T2DM in obese men was 7-fold higher, while in obese women it was 12-fold higher (Wilding, 2014). It is believed that increased visceral obesity is more relevant to development of diabetes than increased body mass index. This is seen in diabetic patients within normal weight range who exhibit visceral obesity (Kolb and Martin, 2017). Diet also has its effects; low fiber content and a high glycemic index increase the risk; while certain fatty acids affect insulin resistance. Vitamin D plays a role in the production and release of insulin in the beta cells of pancreas via 1-alpha-hydroxylase and vitamin D receptor. It also controls membranous calcium flux in beta cells and peripheral target tissues, therefore influencing insulin sensitivity. The negative effect vitamin D deficiency has on glucose tolerance and insulin secretion suggests supplements as a way to decrease the risk of T2DM development (Wu *et al.*, 2014).

High physical activity has been associated with decreased risk of an estimated 30% compared with low physical activity. The positive effects on insulin sensitivity and glycemic control were found not only in T2DM patients but in non-diabetic controls as well (Kolb and Martin, 2017). Changes in lifestyle (either in diet, weight, physical activity or all three) reduce the incidence of diabetes by 51% and reduce retinopathy in T2DM patients by 47% (Wilding, 2014).

Among the medical conditions that are relevant is gestational diabetes which increases the risk 7-fold in women. Metabolic syndrome and hypertension have also been known to

increase the risk of T2DM (Bellou *et al.*, 2018). Smoking (both passive and active) has been linked with diabetes, and high incidence rates were found in current and former smokers, although former smokers were at a lower risk (Pan *et al.*, 2015). Smoking has been linked to changes in body composition, specifically, in adverse fat distribution caused by central and peripheral nicotine signaling. Compared with non-smokers, smokers had higher waist-to-hip ratios that increase with the quantity and duration of smoking; they also had higher visceral adipose to subcutaneous adipose ratios. Glucose tolerance and insulin sensitivity are negatively affected and the function of beta cells is impaired (Maddatu, Anderson-Baucum and Evans-Molina, 2017).

1.1.3. Pathogenesis

The pathogenesis of T2DM is characterized by mainly two things: insulin resistance and pancreatic beta cell dysfunction. Insulin resistance is when the target tissues don't respond to insulin. It is believed to be due to phosphorylation of IRS-1 at serine/threonine positions, leading to a weakened insulin signal (Sears and Perry, 2015).

The most common cause of insulin resistance -especially in developed countries- is obesity; in which levels of plasma free fatty acids (FFA) are increased due to an enlarged adipose tissue and due to a decrease in their clearance. FFA decrease insulin antilipolytic action which increases their levels. Elevated plasma FFA levels can cause a decreased insulin-stimulated glucose uptake; it also inhibits the insulin-mediated suppression of hepatic glucose production. Upon normalization of plasma FFA levels in obese individuals, either diabetic or not, the results show improved insulin sensitivity in both groups (Boden, 2011).

Several mechanisms have been suggested to understand how FFA can affect insulin signaling, high FFA levels in skeletal muscle cause an accumulation of FFA re-esterification products such as diacylglycerol (DAG), which activates protein kinase C (PKC) isoforms β2 and δ, which can impair the tyrosine phosphorylation of IRS-1; therefore causing insulin resistance. Obesity results in a state of low grade inflammation and increasing levels of circulating pro-inflammatory cytokines. Linoleic acid leads to an increased interleukin-6 (IL-6) and tumor necrosis factor (TNF-α) in adipocytes. FFA

were found to activate the pro-inflammatory pathway of the nuclear factor κ light-chain-enhancer of activated B cells (NF-κB pathway), which has a role in insulin resistance. Studies have demonstrated that NF-κB depends on activation of IκB kinase complex (IKK), which in turn depends on PKC activation by DAG. Another study suggests that activation of IKK and NF-κB is mediated by toll like receptor-4 (TLR-4), which has a role in development of immunity and production of pro-inflammatory cytokines (Boden, 2011).

Beta cell dysfunction is present in both T1DM and T2DM. In the latter, the defect is present at the time of diagnosis and worsens with progression of the disease and deteriorating glycemic control. High plasma glucose levels in T2DM simulate insulin secretion, and if insulin sensitivity decreases; insulin secretion will increase to keep glucose levels within the normal range. This will lead to hyperinsulinemia; a characteristic of T2DM. Eventually, beta cells will no longer be able to secrete enough insulin to compensate the decreased insulin sensitivity (Saisho, 2015).

Both beta cell function and mass are decreased in T2DM patients. In people with impaired glucose tolerance and T2DM; a decreased function of 80% was reported. Decreased beta cell mass is also found in T1DM; although the etiology is different. A study has shown that it is the increased rate of beta cells apoptosis, rather than a decrease in their replication, is responsible for the decreased beta cell mass in T2DM. Glucotoxicity, lipotoxicity, endoplasmic reticulum (ER) stress, oxidative stress and inflammation have been suggested as possible mechanisms that induce apoptosis (Saisho, 2015).

Chronic hyperglycemia has negative effects on beta cell function and can lead to irreversible damage to the production and secretion of insulin. Eventually, glucotoxicity leads to cell apoptosis. One possible explanation is that in long-term hyperglycemia, there is a gradual loss of insulin gene expression. Another explanation is ER stress, which occurs due to an increased demand on ER to produce proinsulin for insulin secretion, leading to an accumulation of unfolded proteins. It has also been suggested that chronic hyperglycemia causes oxidative stress by increasing the metabolic flux into the mitochondria and resulting in an excessive production of reactive oxygen species. The

calcium ion homeostasis is disrupted, which impacts the insulin secretion pathway. In lipotoxicity, there is an accumulation of fatty acid metabolites in beta cells. When paired with hyperglycemia; it could lead to diminished insulin gene expression, negatively affecting the beta cell function (Cernea and Dobreanu, 2013).

1.1.4. Diagnosis

The sign and symptoms of diabetes develop gradually over the years and tend to go unnoticeable or ignored in the early stages. This poses a challenge because early detection and control of hyperglycemia could help prevent various complications, especially vascular ones. Common symptoms include polydipsia, polyphagia and polyuria. These develop in case of high levels of hyperglycemia. Other warning signs include unexplained weight loss, frequent fatigue, irritability and dry mouth (Ramachandran, 2014).

The criteria for the diagnosis of diabetes include the following laboratory tests: fasting plasma glucose (FPG) value is above 126 mg/dL; the two-hour plasma glucose (2-h PG) value after a 75-g oral glucose tolerance test (OGTT) is above 200 mg/dL; and the glycated hemoglobin (HbA1C) value is above 6.5%. These tests may also be used to detect prediabetes. The 2-h PG is more diagnostic of diabetes in comparison with FPG and HbA1C. However, using the HbA1C test is more favorable for it has a greater pre-analytical stability and lesser disturbances in illness and stress; it also doesn't require fasting. It should be taken in consideration that hemoglobin glycation is affected by other factors such age, ethnicity and hemoglobinopathies. Repeating a test for confirmation is not required in case of a hyperglycemic crisis or if the patient displays symptoms of hyperglycemia with a random plasma glucose value above 200 mg/dL. However, because laboratory tests have variability in their pre-analytic and analytic phases, misleading results remain a possibility. This is probable in improper centrifugation of glucose samples in FPG and 2-h PG tests (Association, 2017).

1.1.5. Complications

People with T2DM are more likely to develop cardiovascular disease (CVD) in comparison with non-diabetic people. The probability of developing coronary artery

disease and myocardial infarction increases 2-to-4 folds, the mortality rate is also increased; the percentage of T2DM patients above 65 years old who die from CVD is 70%. A possible explanation of this relation is how insulin resistance and hyperglycemia can lead to atherogenesis by promoting inflammation, increasing oxidative stress and eventually causing endothelial damage. Platelet activity is enhanced. Interleukins such as IL-1β and IL-6, as well as TNF- α were found to be involved (De Rosa *et al.*, 2018). Associated conditions include hypertension, dyslipidemia and obesity; and the management of those could help in reducing the risk of CVD in T2DM patients (Martín-Timón, 2014).

Diabetes also contributes in nephropathy that develops due to hyperglycemia, formation of arachidonic acid metabolites as well as hemodynamic adversities. Diabetic nephropathy is found in 20 to 30% of both T1DM and T2DM patients; chronic kidney disease and end-stage renal disease are most commonly caused by diabetes. Other diseases of the urinary tract and the renal parenchyma are also seen (Shahbazian and Rezaii, 2013).

Uncontrolled diabetes is associated with loss of sensation in peripheral neuropathy (prevalence of 0.003 to 2.8%) and ischemia due peripheral arterial disease (prevalence of 0.01 to 0.4%). One or both of these conditions can cause foot ulcers. Foot infection and other foot diseases are likewise found in diabetes, affecting 6% of the patients. The prevalence of those requiring an amputation is between 0.03 and 1.5%. Amputations often start out as ulcers (Mishra *et al.*, 2017).

Nearly 100 million individuals are affected by diabetic retinopathy (DR), and it is considered the most common microvascular complication. Visual impairment associated with DR increased by 64% between 1990 and 2010, and DR-related blindness also increased by 27%. DR is starts as a non-proliferative diabetic retinopathy (NPDR) and becomes a proliferative diabetic retinopathy (PDR) in its later stages. NPDR includes microaneurysms and retinal hemorrhages, this leads to hypoxia, which stimulates the expression of the growth factors of angiogenesis. Renal neovascularization is the characteristic of PDR, hemorrhage or retinal detachment eventually result in vision loss (Duh, Sun and Stitt, 2017).

One possibly outcome is the hyperglycemic hyperosmolar state (HHS). It is a condition of severe hyperglycemia and intense dehydration, with altered consciousness and possibly coma. Unlike diabetic ketoacidosis; acidity and levels of ketone bodies are low, and it is more seen in T2DM patients in older age groups. The estimated mortality rate is 20% especially in those presented with coma. Infections, stroke and myocardial infarctions have been associated with HHS (Pasquel and Umpierrez, 2014).

1.2. Thyroid gland

The thyroid is a gland that secretes the iodothyronines: thyroxine (T4) and triiodothyronine (T3). The thyroid hormones affect the body in many ways; the force of heart contraction is increased, the heart rate, stroke volume and cardiac output are also increased. The peripheral vascular resistance is decreased as a result of dilated blood vessels. Thyroid hormones also cause an increase in basal metabolic rate and the uptake of glucose and fatty acids, as well as increased oxidation, resulting in an enhanced thermogenesis. This explains heat intolerance that occurs in hyperthyroidism. Nerve system is stimulated causing increased alertness attentiveness and the peripheral reflexes; as well as the gastrointestinal motility. Another effect these hormones have is on the endocrine system, including inhibition of prolactin production and the regulation pituitary function and growth hormone production. They also play a role in the regulation of spermatogenesis and ovulatory cycle (Armstrong and Fingeret, 2018).

The iodothyronines are formed inside the thyroid cells from thyroglobulin, which is then paired with iodine in the follicular lumen forming iodinated thyroglobulin. The thyroid gland mainly secretes T4, which is inactive, nearly 90% of the secretion. The remaining 10% are T3, which is active. T4 is converted to T3 in the peripherals by type 1 deiodinase (in tissues like liver or kidneys), type 2 deiodinase (in the brain) and type 3 deiodinase, the latter converts T4 to reverse T3, which is inactive (Armstrong & Fingeret, 2018).

1.2.1. Hyperthyroidism

Hyperthyroidism is the excessive synthesis and secretion of thyroid hormones. It is characterized by low thyroid-stimulating hormone (TSH) and high T3 and T4 concentrations. Its occurrence is seen more in women and increases with age; it is also

more likely in areas with iodine deficiency. The incidence of mild hyperthyroidism is decreasing since the beginning of salt iodization programs (De Leo, Lee and Braverman, 2016).

Hyperthyroidism affects many organs; its sign and symptoms could be cardiovascular: such as palpitation and tachycardia, or nervous: such as fatigue, nervousness, anxiety, tremor, poor concentration, general weakness and disturbed sleep. Heat intolerance, sweating and weight loss are also common. Certain causes of the hyperthyroidism have other sign and symptoms; ophthalmopathy, dermopathy, and acropachy are found in Graves' disease. In nodular goiter; oesophageal and tracheal compression causes dysphagia and orthopnoea. Older patients are more like to develop cardiovascular complications; particularly atrial fibrillation accompanied by embolic stroke. Patients with hyperthyroidism have an increased mortality rate, mainly due to heart failure (De Leo, Lee and Braverman, 2016).

Subclinical hyperthyroidism is when there are low levels of TSH and normal levels of T3 and T4. It can be either low yet detectable; where TSH is between 0.1-0.4 mIU/L, or it could be even less than 0.1 mIU/L. Subclinical hyperthyroidism is mostly seen in thyroid hormone replacement therapy, with a prevalence of nearly 20%. It could also be due Graves' disease, function thyroid adenomas or multinodular toxic goiter. Subacute thyroiditis and postpartum thyroiditis may cause a transient decrease in TSH levels (Donangelo and Suh, 2017).

Graves' disease is a common cause of primary hyperthyroidism especially among young females. It is caused by autoantibodies acting against TSH receptors, the thyroid gland is enlarged and hypervascular without apparent nodularity. In toxic nodular goiter, the gland is enlarged and has multiple nodules, it is a condition seen in both males and females; mostly in older age groups. Hyperthyroidism could also be due to functional thyroid tumors, such as benign hyperfunctional adenomas, which is found usually in women at older age. The cause of hyperthyroidism is rarely a malignant tumor, but if it is, it could be a follicular carcinoma or a papillary carcinoma (LiVolsi and Baloch, 2018).

The causes of hyperthyroidism may be secondary, due to a pituitary gland lesion, such as a multifocal thyrotroph hyperplasia, or it may be tertiary, due to a lesion of the hypothalamus –tumors for example-which by increasing thyrotropin releasing hormone (TRH) can stimulate TSH secretion, and in turn increase levels of thyroid hormones. These are rare and represent less than 1% of hyperthyroid patients. Struma ovarii, which is the presence of thyroid tissue in the majority of a teratoma in an ovary; called monodermal teratoma, can also be a cause of hyperthyroidism. In non-hyperthyroid thyrotoxicosis, the thyroid hormones stored inside colloid are released into the circulation due to rapid destruction of follicular epithelium. One cause of this destruction is subacute thyroiditis, also known as granulomatous thyroiditis or de quervain thyroiditis; it is linked to thyroid viral infection and results in anterior neck pain. Upon healing, hypothyroidism occurs because the thyroid can't use iodide to synthesize more hormones (LiVolsi and Baloch, 2018).

1.2.2. Hypothyroidism

Hypothyroidism is thyroid hormone deficiency. It is categorized as primary or secondary according to its etiology, and as clinical or subclinical according to its severity. In primary hypothyroidism, T3 and T4 are decreased while TSH is increased. On the other hand, in secondary hypothyroidism, T3 and T4 are decreased while TSH is normal or decreased. In subclinical hypothyroidism, T3 and T4 are normal and TSH is increased. Sign and symptoms include fatigue, cold intolerance, dry skin, voice hoarseness, periorbital edema and weight gain. In clinical hypothyroidism, the symptoms are more apparent. In severe cases, other manifestations are possible, including congestive heart failure, pleural effusion, coagulation disorders, depression, seizures, psychosis and coma. Primary hypothyroidism is when the problem is with the thyroid gland itself that causes decreased secretion and synthesis. Chronic autoimmune thyroiditis makes up nearly half the cases in hypothyroidism. It affects women 3 to 5 times more than men, usually seen in middle age and older. Infection with agents such as Epstein- Barr virus was associated with autoimmune thyroiditis in children. Also; infection with parvovirus B19 has been linked to Hashimoto's thyroiditis in children (Kostoglou-Athanassiou and Ntalles, 2010).

In 5-10% of women, antithyroid antibodies increase after delivery during the first year. This condition is known as postpartum thyroiditis. It either manifests as a mild hyperthyroidism that may become hypothyroidism or as hypothyroidism only. Hypothyroidism in partial thyroidectomy that was performed due to hyperthyroidism could be either clinical (17%) or subclinical (51.3%). Thyroid agenesis and dysgenesis as well as any disorder that affects the synthesis of thyroid hormone could result in hypothyroidism in children and infants. It may also develop in infants whose mothers underwent antithyroid therapy during pregnancy. Any disorder in the pituitary gland that results in a decreased secretion of TSH leads to secondary hypothyroidism, also called central hypothyroidism (Kostoglou-Athanassiou and Ntalles, 2010).

Subclinical hypothyroidism is more common among middle-age and older age groups. It is an asymptomatic disorder, and diagnosis is confirmed when TSH levels are over 4 mIU/L. TSH levels between 4 and 10 mIU/L are found in 90% of subclinical patients. If they increase above 10 mIU/L, symptoms and complications may start to show, and replacement therapy is advised (Cojić and Cvejanov-Kezunović, 2017).

1.2.3. Thyroid autoantibodies

In cases of autoimmune thyroid diseases, several antibodies can be detected in the serum. The most common among those are anti-thyroid peroxidase (anti-TPO) and anti-thyroglobulin (anti-TG) antibodies. Fewer cases include antibodies against carbonic anhydrase-2, sodium iodide symporter, T3 and T4. Thyroid peroxidase is an intracellular membrane-bound enzyme that catalyzes the iodination of the tyrosine residue of the thyroglobulin molecules forming monoiodotyrosine and diiodotyrosine. Anti-TPO can be immunoglobulin (Ig) A or G, but mostly the latter, specifically, IgG1 and IgG4. In autoimmune patients, anti-TPO antibodies can fix complement, cause damage to thyroid cells and interfere with enzymatic activity. They are more common than anti-TG in autoimmune thyroid disease and exist in 90-95% of its patients. Regarding thyroglobulin, 1-6 epitopes out of 40 are believed to be immunogenic. Anti-TG antibodies are mostly in IgG4 class, with lower levels of IgA. They do not fix complement and do not cause direct thyroid cell damage. Studies reported a prevalence of 60-80% of anti-TG antibodies in autoimmune thyroid disease patients (Fröhlich and Wahl, 2017). It has been reported that

the prevalence of anti-TPO and anti-TG antibodies in adults were 13% and 11.5%, respectively (Amouzegar *et al.*, 2017).

1.3. Relationship between type 2 diabetes mellitus and thyroid dysfunction

Diabetes mellitus and thyroid are associated with one another. Several studies have been conducted to estimate the prevalence of thyroid dysfunction in T2DM. One study in Greek diabetic patients found a prevalence of 12.3% (Papazafiropoulou, 2010), while another found it to be 16% in Saudi diabetic patients (Akbar, Ahmed and Al-Mughales, 2006). A similar study took place in Jordan, where the prevalence was 12.5% (Radaideh *et al.*, 2004). Thyroid hormones affect glucose regulation while diabetes influences thyroid function. Hyperthyroidism promotes hyperglycemia in several ways; the degradation of insulin is increased, therefore reducing its half-life. Hyperthyroidism also promotes glucose gastrointestinal absorption and increases the glucose transporter-2 (GLUT-2) plasma membrane concentration in liver cells therefore increasing the hepatic glucose output. Hepatic gluconeogenesis is increased due to FFA generated by enhanced lipolysis, there is also an overproduction of lactate in non-oxidative glucose disposal (Hage, Zantout and Azar, 2011).

In hypothyroidism, glucose gastrointestinal absorption, gluconeogenesis, hepatic glucose output and glucose disposal are reduced. Insulin resistance has been linked with subclinical hypothyroidism and leads to insulin secretion. Also in subclinical hypothyroidism, GLUT-2 expression is diminished (Wang, 2013). The risk of diabetic nephropathy in T2DM patients is increased in the presence of subclinical hypothyroidism and the diabetic retinopathy is more severe (Hage, Zantout and Azar, 2011).

Rational

- Thyroid dysfunction and diabetes are both disorders of the endocrine system and are believed to influence one another.

General objectives

-To observe the relation between thyroid dysfunction and T2DM.

Specific objectives

- To evaluate the relation between the following: age, gender, body mass index (BMI), duration of diabetes, usage of oral hypoglycemic drugs and insulin, hypertension and the development of thyroid dysfunction in T2DM patients.

- To determine the frequency of thyroid autoimmunity in T2DM patients with or without thyroid dysfunction.

Aim of the study

This study aims to assess the frequency and pattern of thyroid dysfunction in T2DM patients.

Hypotheses

- Thyroid status is affected in diabetic patients.

- The gender, age, BMI, duration of diabetes, drug therapy and presence of hypertension play a role in developing thyroid dysfunction.

- The prevalence of thyroid autoimmunity is higher in diabetic patients.

- The prevalence of thyroid autoimmunity is higher in female patients.

2. Methodology

2.1. Study design

2.1.1. Type of study

It is an observational case control study.

2.1.2. Study population

The population consisted of 90 individuals of the adult age. Among them 71 patients who've been diagnosed with T2DM from the Benghazi Diabetic Center. The control group included 19 volunteers.

Exclusion criteria included previous diagnosis with thyroid dysfunction, acute illness and pregnancy.

2.1.3. Ethical consideration

The study was approved by the administration of the Libyan International Medical University and the Benghazi Diabetic Center. Participants were asked for consent and they were informed of the results.

2.2. Sampling techniques

2.2.1. Sample collection

The collection took place from May 28th to July 25th 2019. A questionnaire was performed to obtain data, including age, gender, weight, height, smoking habits, family history, duration of diabetes, history of drug therapy, and history of other illness.

Venous blood samples of 3 milliliter were obtained from the patients after an overnight fasting. This was done with the help of the staff in Benghazi Diabetic Center; each sample was collected from each patient in a plain serum tube.

2.2.2. Sample preparation and storage

The blood samples underwent centrifugation at 4000 RPM for 5 minutes to obtain serum. The serum was separated using a 500 µL pipette and stored in 1.5 milliliter Eppendorf tubes in a temperature of – 20 ^{o}C.

2.3. Biochemical assays

Five ELISA tests were carried out between July 29th and August 1st using EMP microplate reader M201 in the laboratory of LIMU. AccuDiag ELISA kits were used for the following tests: TSH (DA318022001), T3 (LOT: DA318011103) and free T4 (LOT: DAE12K1116). Euroimmun ELISA kits were used for the antibodies: anti-TPO (LOT: E171110BD) and anti-TG (LOT: E171114AA). Each kit for these tests included 96 wells.

The first test was to evaluate TSH levels in the serum. The assay system uses monoclonal anti-TSH antibody in the microtiter wells (solid phase) and anti-TSH antibody in the antibody-enzyme conjugate solution (horseradish peroxidase). TSH is placed between the solid phase and the enzyme-linked antibodies. The wells were incubated for 60 minutes then washed to remove unbound labeled antibodies. After adding a TMB substrate and incubating for 20 minutes, the contents gave a blue color, which after adding a stop solution turned to yellow. The intensity of the color was measured using ELISA at 450nm.

The second test was to evaluate T3 levels in the serum. The sample and a constant amount of enzyme-conjugated T3 were added in microtiter wells that contain anti-T3 antibody. The wells were incubated for 60 minutes; during which the T3 and the conjugated T3 compete for the binding sites on the anti-T3 antibody. The wells were then washed to remove unbound T3 conjugate. TMB solution was added and incubated for 20 minutes, giving a blue color that turned yellow after adding a stop solution. The intensity of the color was measured using ELISA at 450nm.

The third test performed was to evaluate free T4. The sample was placed in a microtiter well containing the antibody and an enzyme-antigen conjugate was added. This was followed by a 60 minute incubation during which a competitive reaction occurs for

limited binding sites. The wells underwent washing to remove unbound T4. The enzyme activity in the antibody-bound fraction is inversely proportional to the free T4 concentration.

The fourth test was to determine the presence of anti-TPO antibodies in the serum. The wells are coated with thyroid peroxidase that reacts with the antibodies in the diluted sample during incubation. An enzyme conjugate (peroxidase-labelled anti-human IgG) promotes a color reaction where the intensity of the color is proportional to the antibody concentration.

The last test was to determine the presence of anti-TG antibodies. The wells are coated with thyroglobulin that reacts with the antibodies in the diluted sample during incubation. Peroxidase-labelled anti-human IgG promotes a color reaction where the intensity of the color is proportional to the antibody concentration. In the last two tests, the results were determined using the semi-quantitative analysis by calculating the ratio of the absorbance of the sample over the absorbance of calibrator-2. The result is positive if the ratio equals 1 or above. If it's below 1, the result is negative.

2.4. Statistical analysis

Minitab was used to analyze the obtained data. Chi‑square test and 2-sample t test were used to compare the variables between groups. Quantitative variables were expressed as mean, while categorical variables were expressed as percentages. Difference was considered significant if p-value was less than 0.05.

3. Results

3.1. Sample distribution

3.1.1. Gender

In the T2DM patients (n=71), 45.07% were male (n=32) and 54.93% were female (n=39) (Figure 1). In the control group (n=19), 36.84% were male (n=7) while 63.16% were female (n=19) (Figure 2).

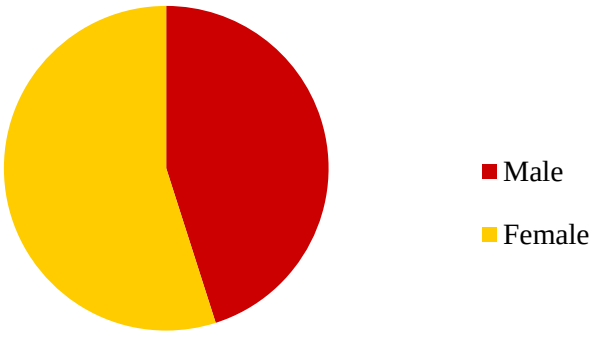

Figure 1: distribution of gender among T2DM group

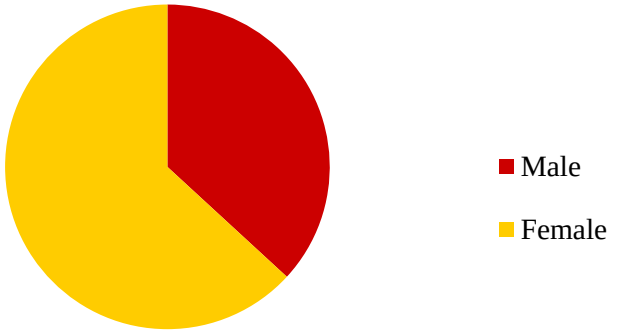

Figure 2: distribution of gender among control group

3.1.2. Age

The mean age (in years) for the patients was 58.056, among those patients 1.4% (n=1) belonged to the age group of <40 years, 19.72% (n=14) belonged to the age group of 40-49 years, 32.39% (n=23) belonged to the age group of 50-59 years, 29.58% (n=21) belonged to the age group of 60-69 years, and 16.9% (n=12) belonged to the age group of 70 years or above (Figure 3). The mean age (in years) for the control group was 38.579, among them 57.89% (n=11) belonged to the age group of <40 years, 15.79% (n=3) belonged to the age group of 40-49 years, and 26.32% (n=5) belonged to the age group of 50-59 years (Figure 4).

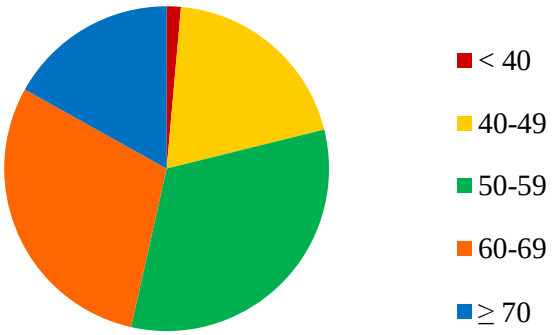

Figure 3: distribution of age groups among T2DM group

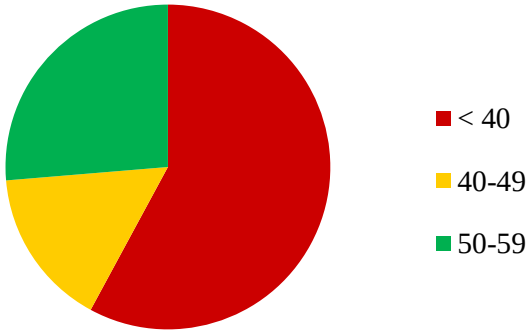

Figure 4: distribution of age groups among control group

3.1.3. BMI

The mean BMI for the patients was 31.323, among them 11.28% (n=8) belonged to normal range group (BMI between 18-24.9), 35.21% (n=25) belonged to the overweight group (25-29.9), 33.8% (n=24) belonged to the obese class I group (30-34.9), 14.08% (n=10) belonged to the obese class II group, and 5.63% (n=4) belonged to the obese class III group (40 or above) (Figure 5). The mean BMI for the control group was 26.789, among them 26.32% (n=5) belonged to the normal range group, 52.63% (n=10) belonged to the overweight group, 21.05% (n=4) belonged to the obese class I group (Figure 6).

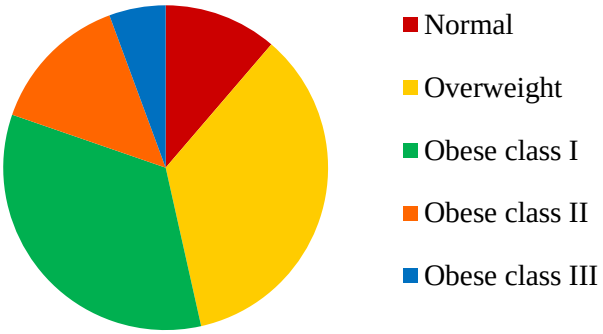

Figure 5: distribution of BMI groups among T2DM group

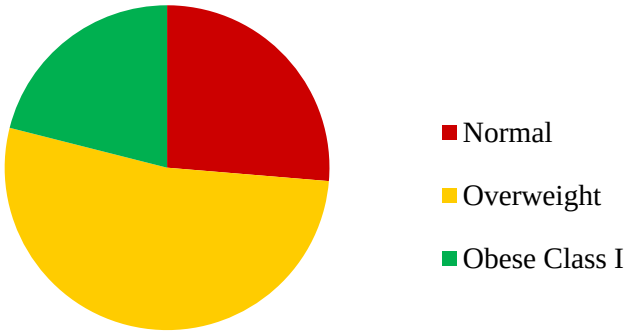

Figure 6: distribution of BMI groups among control group

3.1.4 Duration of diabetes

The patients according to the duration of the diabetes were categorized as the following: 5.64% (n=4) had duration of <1 year, 11.27% (n=8) had duration of 1-5 years, 28.17% (n=20) had duration of 6-10 years, 15.49% (n=11) had duration of 11-15 years, 23.94% (n=17) had duration of 16-20 years, 7.04% (n=5) had duration of 21-25 years, and lastly, 8.45% (n=6) had duration of >25 years (Figure 7).

Figure 7: distribution of duration groups among T2DM group

3.1.5. Drug therapy

Among the patients, 2.82% (n=2) took no treatment, 19.72% (n=14) were on oral hypoglycemic agents, 14.08% (n=10) were on insulin, and 63.38% (n=45) were on both oral hypoglycemic agents and insulin (Figure 8).

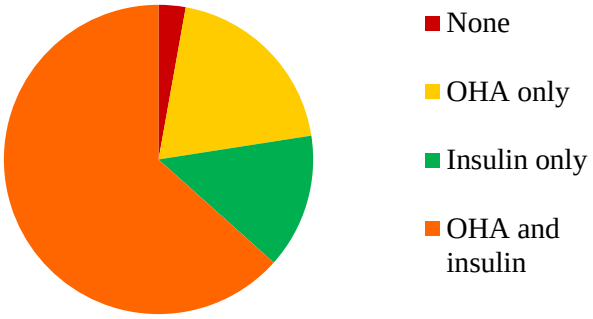

Figure 8: distribution of drug therapy groups among T2DM group

3.1.6. Hypertension

None of the individuals in the control group were previously diagnosed with hypertension (n=0); while among the patients, 40.85% (n=29) were diagnosed with hypertension, and the remaining 59.15% (n=42) were not (Figure 9).

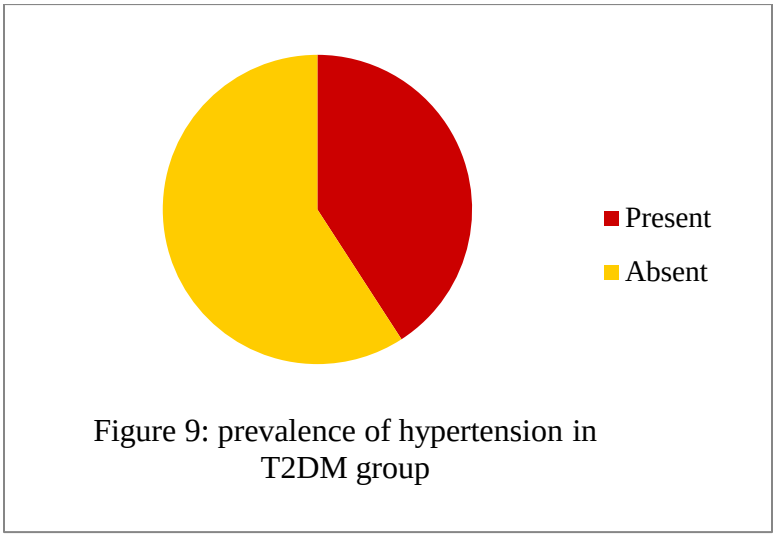

Figure 9: prevalence of hypertension in T2DM group

3.2 Thyroid dysfunction among T2DM patients and controls

3.2.1. Thyroid function

Among the T2DM patients participating in the study (n=71), 8.45% (n=6) of the patients showed subclinical hypothyroidism, while the remaining 91.55% (n=65) were euthyroid (Figure 10). In the control group, 10.5% (n=2) showed subclinical hypothyroidism, and 89.5% (n=17) were euthyroid (Figure 11). None of the participants in either group showed hypothyroidism, hyperthyroidism or subclinical hyperthyroidism.

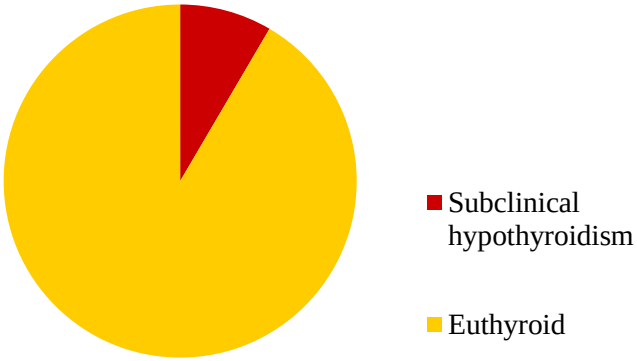

Figure 10: prevalence of thyroid dysfunction in T2DM group

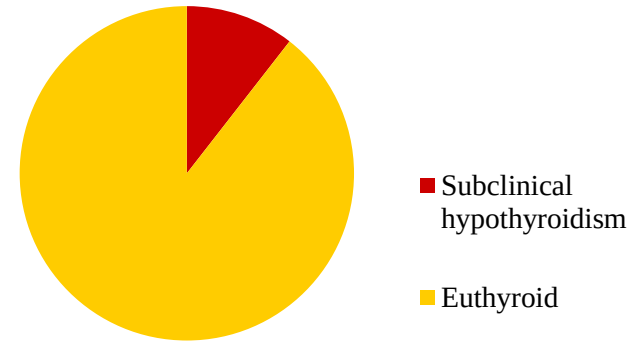

Figure 11: prevalence of thyroid dysfunction in control group

3.2.2. Thyroid dysfunction and gender

The prevalence of thyroid dysfunction according to gender was 50% male (n=3) and 50% female (n=3), those who were euthyroid were 44.62% (n=29) male and 55.38% (n=36) female. In the control group, both with thyroid dysfunction were female 100% (n=2), while the euthyroid were 41.18% (n=7) male and 58.82% (n=10) (Figure 12). There was no statistical significance between thyroid status and gender in neither diabetic patients nor controls (Table 4, p-value > 0.05).

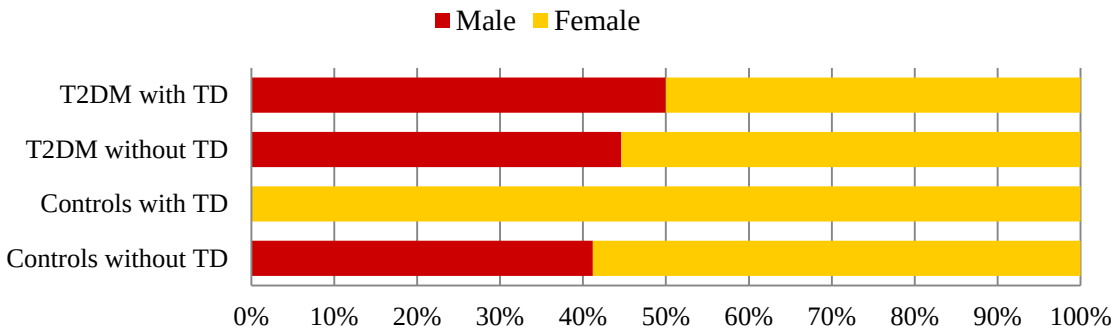

Figure 12: prevalence of thyroid dysfunction according to gender

3.2.3. Thyroid dysfunction and age

According to their age groups, the patients with thyroid dysfunction were as follows: 33.33% (n=2) of patients belonged to the age group of 40-49 years, 33.33% (n=2) of patients in the age group of 50-59 years, 16.67% (n=1) of patients in the age group of 60-69 years and 16.67% (n=1) of patient in the age group of 70 years or above. The prevalence of euthyroid patients was as follows: 1.54% (n=1) were <40 years, 18.46% (n=12) were 40-49 years. 32.31% (n=21) were 50-59 years, 30.77% (n=20) were 60-69 years and 16.92% (n=11) were > 70 years (Figure 13). The difference between the two groups was not of statistical significance (Table 1). In the control group with thyroid dysfunction, 50% (n=1) belonged to the age group of < 40 years and 50% (n=1) belonged to the age group of 40-49 years. The euthyroid controls were as follows: 58.82% (n=10) were < 40 years, 11.76% (n=2) were 40-49 years and 29.42% (n=5) were 50-59 years (Figure 13). The difference between the two groups was not of statistical significance (Table 1). There was also no statistical significance between thyroid status and age groups in neither diabetic patients nor controls (Table 4, p-value > 0.05).

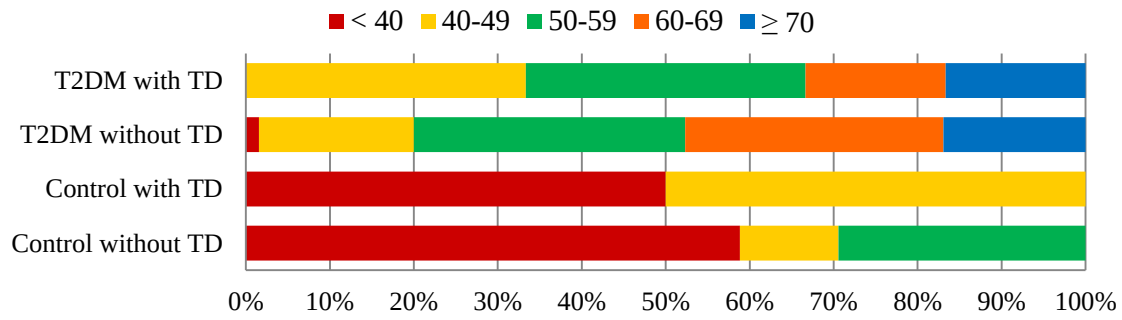

Figure 13: prevalence of thyroid dysfunction according to age groups

Table 1: Age in T2DM patients and controls, with and without thyroid dysfunction

	T2DM patients with TD	T2DM patients without TD	Controls with TD	Control without TD
Age (years), mean	56.17	58.23	38.5	38.6

3.2.4. Thyroid dysfunction and BMI

In regards to their BMI group, the patients with thyroid dysfunction were as follows: 16.67% (n=1) belonged to the normal weight group, 33.33% (n=2) belonged to the overweight group, 33.33% (n=2) belonged to the obese class I group and 16.67% (n=1) belonged to the obese class II group. Regarding the euthyroid patients: 10.77% (n=7) were normal weight, 35.38 (n=23) were overweight, 33.85 (n=22) were obese class I, 13.85% (n=9) were obese class II and 6.15% (n=4) were obese class III (Figure 14). The difference between the two groups was not of statistical significance (Table 2). In the control group, 50% (n=1) patient belonged to the overweight group and 50% (n=1) patient belonged to the obese class I group. In the euthyroid controls, 29.41% (n=5) were normal weight, 52.94% (n=9) were overweight and 17.65% (n=3) were obese class I (Figure 14). The difference between the two groups was not of statistical significance (Table 2). There was also no statistical significance between thyroid status and BMI groups in neither diabetic patients nor controls (Table 4, p-value > 0.05).

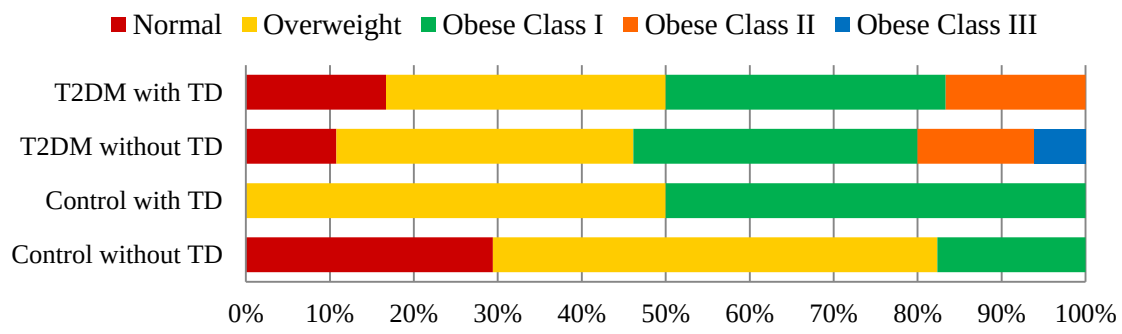

Figure 14: prevalence of thyroid dysfunction according to BMI groups

Table 2: BMI in T2DM patients and controls, with and without thyroid dysfunction

	T2DM patients with TD	T2DM patients without TD	Controls with TD	Control without TD
BMI, mean	30.27	31.42	31.35	26.25

3.2.5. Thyroid dysfunction and duration of diabetes

In the group patients with thyroid dysfunction, 16.67% (n=1) had a duration of 6-10 years, 33.33% (n=2) had a duration of 11-15 years, 33.33% (n=2) had a duration of 16-20 years and 16.67% (n=1) had a duration of 21-25 years. In the euthyroid group, 6.15% (n=4) had a duration of <1 year, 12.31% (n=8) had a duration of 1-5 years, 29.23% (n=19) had a duration of 6-10 years, 13.85% (n=9) had a duration of 11-15 years, 23.08% (n=15) had a duration of 16-20 years, 6.15% (n=4) had a duration of 21-25 years and 9.23% (n=6) had a duration of > 25 years (Figure 15). The difference between the two groups was not of statistical significance (Table 3). There was also no statistical significance between thyroid status and duration groups in diabetic patients (Table 4, p-value > 0.05).

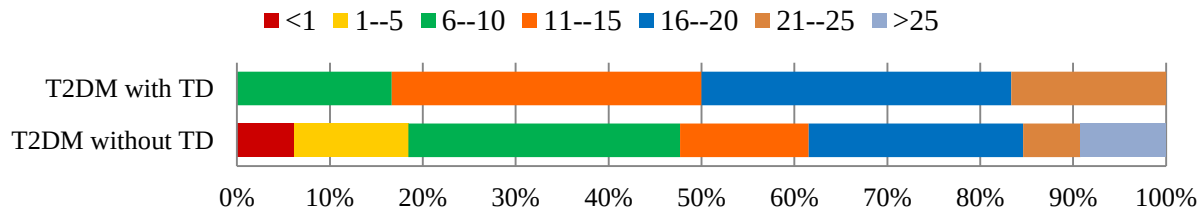

Figure 15: prevalence of thyroid dysfunction according to duration groups

Table 3: Duration of diabetes in T2DM patients, with and without thyroid dysfunction

	T2DM patients with TD	T2DM patients without TD
Duration (years), mean	16.67	13.38

3.2.6. Thyroid dysfunction and drug therapy

It was found that 83.33% (n=5) out of diabetic patients with thyroid dysfunction were on both insulin and oral hypoglycemic agents and 16.67% (n=1) of patients were on insulin only. In euthyroid group, 3.08% (n=2) took no treatment, 21.53% (n=14) were on oral hypoglycemic agents, 13.85% (n=9) were on insulin and 61.54% (n=40) were on both oral hypoglycemic drugs and insulin (Figure 16). There was also no statistical significance between thyroid status and drug therapy in diabetic patients (Table 4, p-value > 0.05).

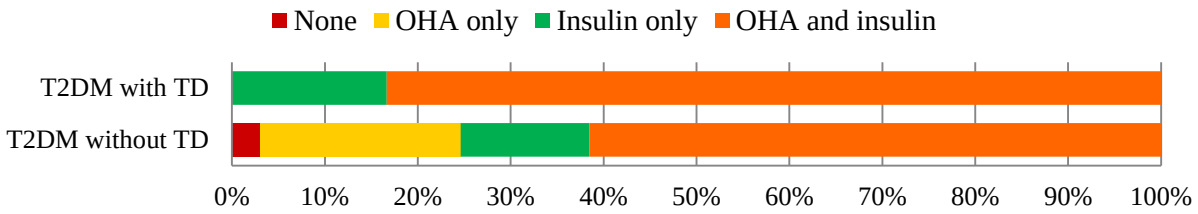

Figure 16: prevalence of thyroid dysfunction according to drug therapy

3.2.7. Thyroid dysfunction and hypertension

It was also found that among the diabetic patients with thyroid dysfunction, 50% (n=3) had been previously diagnosed with hypertension, while the remaining 50% (n=3) were not. In the euthyroid group, 40% (n=29) had hypertension and 60% (n=39) did not (Figure 17). There was also no statistical significance between thyroid status and hypertension in diabetic patients (Table 4, p-value > 0.05).

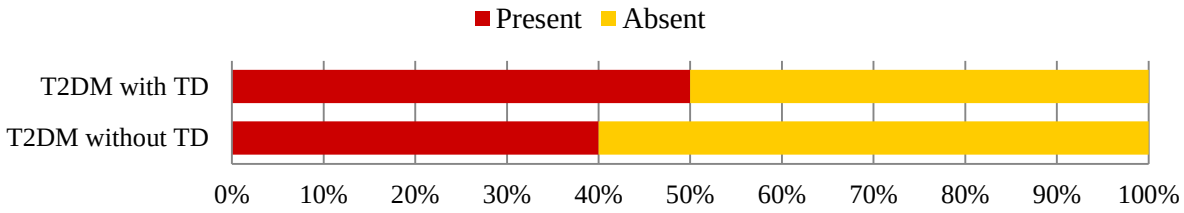

Figure 17: prevalence of thyroid dysfunction according to hypertension

Table 4: Correlation of thyroid status with gender, age, BMI, duration of diabetes, drug therapy and presence of hypertension

	Thyroid status	
Parameter	P-value	
	T2DM patients	Controls
Gender	0.8	0.253
Age	0.897	0.321
BMI	0.965	0.476
Duration of diabetes	0.62	/
Drug therapy	0.589	/
Hypertension	0.634	/

3.3. Thyroid autoantibodies

Both groups were tested for the presence of thyroid autoantibodies. Among the patients group, 14.08% (n=10) had thyroid autoimmunity, 12.68% (n=9) were found to be positive for anti-TPO, and 2.82% (n=2) for anti-TG. Among the control group, 15.79% (n=3) had thyroid autoimmunity, 15.79% (n=3) were positive for anti-TPO and 5.26% (n=1) for anti-TG (Figure 18).

Among the diabetic male patients, 6.25% (n=2) had thyroid autoimmunity, 6.25% (n=2) tested positive for anti-TPO and 3.13% (n=1) for anti-TG, and among the female patients, 20.51% (n=8) had thyroid autoimmunity, 17.95% (n=7) tested positive for anti-TPO and 2.56% (n=1) for anti-TG. Among the male controls 14.29% (n=1) had thyroid autoimmunity and tested positive for anti-TPO, and among the females 16.67% (n=2) had thyroid autoimmunity and tested positive for anti-TPO and 8.33% (n=1) for anti-TG (Figure 18).

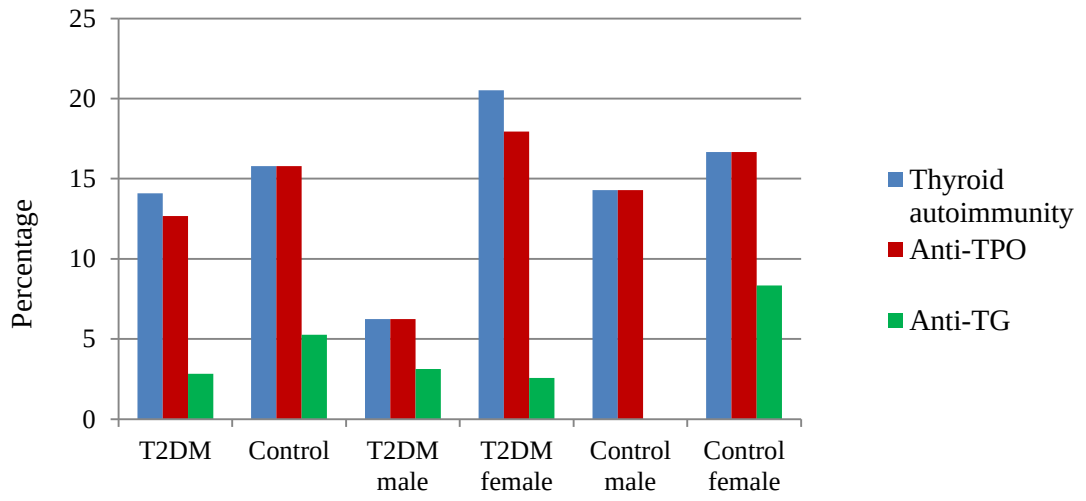

Figure 18: prevalence of thyroid autoantibodies in T2DM and control groups

Among the patients with thyroid dysfunction, the prevalence of thyroid autoimmunity was 33.33% (n=2) and in euthyroid patients it was 12.31% (n=8). In the controls with thyroid dysfunction, the prevalence of thyroid autoimmunity was 50% (n=1) and in euthyroid controls it was 11.76% (n=2) (Figure 19).

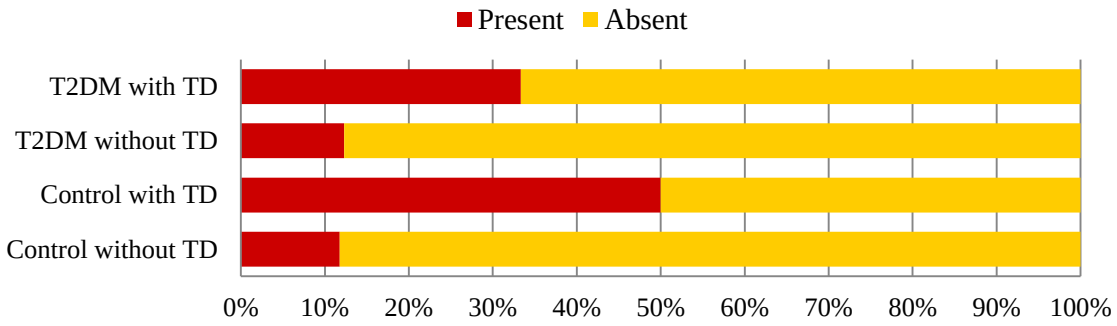

Figure 19: prevalence of thyroid autoimmunity

4. Discussion

Out of the 71 diabetic patients who participated in the study, 8.45% had thyroid dysfunction (Figure 10). This finding is close to a study in Western-Libya where the prevalence was 9.5% (Ahmed Ahmed, 2017). However, it differs from other studies where it was 21.9% in Bangladesh (Kamrul-Hasan *et al.*, 2018), 16% in India (Khurana, Dhoat and Jain, 2016), 14.7% in Brazil (Palma *et al.*, 2013), 13.1% in Saudi Arabia (Hammadi, Aljawi and Alahdal, 2018), 12.3% in Greece (Papazafiropoulou, 2010), 32.4% in Spain (Díez, Sánchez and Iglesias, 2011) and 48% in Argentine (Centeno Maxzud *et al.*, 2016).

Subclinical hypothyroidism was the only thyroid dysfunction among the patients and the controls. It should be noted that in other studies, subclinical hypothyroidism showed the highest prevalence: 5% in Western-Libya (Ahmed Ahmed, 2017), 14.1% in Bangladesh (Kamrul-Hasan *et al.*, 2018), 7.5% in India (Khurana, Dhoat and Jain, 2016), 12% in Brazil (Palma *et al.*, 2013), 12% in Saudi Arabia (Hammadi, Aljawi and Alahdal, 2018) and 10.7% in Spain (Díez, Sánchez and Iglesias, 2011).

The prevalence of male T2DM patients with thyroid dysfunction was the same as the female patients (Figure 12), with no statistical significance (Table 4, p-value > 0.05). The result is different from other publications; where the females had a higher prevalence (Papazafiropoulou, 2010; Díez, Sánchez and Iglesias, 2011; Khurana, Dhoat and Jain, 2016; Ahmed Ahmed, 2017; Telwani *et al.*, 2017; Hammadi, Aljawi and Alahdal, 2018; Kamrul-Hasan *et al.*, 2018).

According to their age, the prevalence of patients with thyroid dysfunction was higher in the age group of 40-49 years and 50-59 years (Figure 13). The relationship was not found statistically significant (Table 4, p-value > 0.05). Certain studies found that thyroid dysfunction did not vary among age groups (Papazafiropoulou, 2010; Kamrul-Hasan *et al.*, 2018). While others observed a higher prevalence in older age groups (Khurana, Dhoat and Jain, 2016; Telwani *et al.*, 2017).

In regards to their BMI group, the prevalence of patients with thyroid dysfunction was higher in the overweight and the obese class I group (Figure 14). This result showed no

statistical significant (Table 4, p-value > 0.05). One study found no significant difference (Díez, Sánchez and Iglesias, 2011), while others observed higher BMI in thyroid dysfunction (Papazafiropoulou, 2010; Khurana, Dhoat and Jain, 2016; Telwani *et al.*, 2017; Kamrul-Hasan *et al.*, 2018).

The prevalence of patients with thyroid dysfunction was higher in the duration groups of 11-15 years and 16-20 years (Figure 15). These were not found significant (Table 4, p-value > 0.05). Similar results were recorded in three studies (Papazafiropoulou, 2010; Díez, Sánchez and Iglesias, 2011; Khurana, Dhoat and Jain, 2016), while one found a significant relation with longer duration (Telwani *et al.*, 2017) and another found it significant when hypertension was present (Hammadi, Aljawi and Alahdal, 2018).

Thyroid dysfunction was mostly seen in diabetic patients who were on both insulin and oral hypoglycemic agents (Figure 16). However, this was not statistically significant (Table 4, p-value > 0.05). Other publications also found the prevalence of thyroid dysfunction highest in patients who were on both insulin and oral hypoglycemic agents (Khurana, Dhoat and Jain, 2016).

The prevalence of hypertensive T2DM patients with thyroid dysfunction was the same as the normotensive patients (Figure 17). The result was not statistically significant (Table 4, p-value > 0.05). In one study, the prevalence was higher in the hypertensive group, but the relation wasn't significant (Khurana, Dhoat and Jain, 2016). Others found no significant relationship between hypertension and thyroid dysfunction in T2DM patients (Papazafiropoulou, 2010; Díez, Sánchez and Iglesias, 2011).

Thyroid autoimmunity was found in 14.08% of all diabetic patients (Figure 18). The results were higher in the Bangladeshi study (32.9%), but it should be noted thyroid microsomal antibodies were tested as well in that study (Kamrul-Hasan *et al.*, 2018). The Brazilian study showed closer results (9.9%) but these were for anti-TPO only (Palma *et al.*, 2013). The Spanish one tested for both anti-TPO and anti-TG antibodies and the findings were the most similar where thyroid autoimmunity was positive in 15.7% in T2DM patients, anti-TPO was positive in 12.6% and anti-TG was positive in 9.7% (Díez,

Sánchez and Iglesias, 2011). Anti-TPO antibodies were found to be positive in 13% of Argentinian diabetic patients (Centeno Maxzud *et al.*, 2016).

In this study, thyroid autoimmunity was seen more among females (Figure 18). The Spanish study also showed similar finding: females (21.5%), males (7.1%) (Díez, Sánchez and Iglesias, 2011). In the Bangladeshi study, the two genders were closer in prevalence: females (34.7%) and males (28.2%) (Kamrul-Hasan *et al.*, 2018). In the current study, the prevalence for thyroid autoimmunity was higher in patients with thyroid dysfunction than in euthyroid patients (Figure 19). The results were higher in Bangladesh where thyroid autoimmunity was present in 59.3% of patients with thyroid dysfunction and 23.5% of euthyroid patients (Kamrul-Hasan *et al.*, 2018). Anti-TG antibodies were tested in Western-Libya, the prevalence was 23.3% in hypothyroidism but none were found in subclinical hypothyroidism (Ahmed Ahmed, 2017).

5.1. Conclusion

The prevalence of thyroid dysfunction in T2DM patients was lower than other studies. However, subclinical hypothyroidism was found to be the most common disorder, similar to other findings. Statistical analysis showed no significant relationship between thyroid dysfunction and the following: gender, age, BMI, duration of diabetes, usage of insulin and oral hypoglycemic agents or hypertension. The prevalence of thyroid autoimmunity was similar in certain studies. Participants with thyroid dysfunction had higher frequencies of thyroid autoimmunity than the euthyroid ones. Thyroid autoimmunity was also more seen in females.

5.2. Recommendations

It is advised that further work is done on the subject of thyroid dysfunction among T2DM patients including:

1. Similar studies should be carried out on a larger scale to include other areas.
2. T1DM should be included in further studies.
3. More investigations and subsequent follow-up should be carried out among participants.

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Appendix

Libyan International Medical University

Faculty of Basic Medical Science

Questionnaire

Number: ..... Date: / / 2019

Age: ..... Gender: Male / Female

Body Mass Index: .....

Smoker: Yes / No

Duration of diabetes: .....

Family history: Yes / No Relation: .....

Insulin / oral hypoglycemic drugs / neither

History of drug therapy: .....

History of illness: .....

FPG ☐ Anti-TPO ☐

T3 ☐ Anti-TG ☐

T4 ☐

TSH ☐

Patient's signature: .....