

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

”وَفَلَا تَنْفَسُوا كَمَا أَنْفَسْتُمْ أَنْفَافًا تَنْبَطِرُونَ“

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Declaration

I hereby declare that this thesis submitted is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to substantial extent has been accepted for award of any other degree of the university or other institute, except where otherwise acknowledgment has been made in the text.

HOSSAM YOUSIF R. QWADER

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Date: Feb. 2007

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Dedication

*I would like to dedicate this work to
all my family: father, mother, wife, children
(Joumana ,Essraa, Yousif ,Moaz , Mohammed),
sister, brothers for their endless support
and motivation.*

Abstract

Autoimmune diseases are manifested in a wide-ranging at all the world and always concomitant with each other. Autoimmune diseases are in most cases characterized by the presence of specific autoantibodies. Diabetes Mellitus type1 as the most common autoimmune disease often associated with other autoimmune diseases. Thyroid autoimmune disease is the most frequently concomitant disease to T1DM.

Aims of the study: to evaluate the existence of anti-Glutamic acid decarboxylase antibodies (GAD) in T1DM patients, and to estimate anti-thyroglobulin antibodies (TG) and anti-Peroxidase antibodies (TPO) as markers of thyroid autoimmune disease in T1DM patients. The study was also interested to assess the association between thyroid antibodies (anti-TG, and anti-TPO) Abs and anti-GAD Abs and assess thyroid diseases among T1DM by tested thyroid releasing hormone (TSH) and free thyroxin (FT4).

Methodology: the sample size was 80 patients, recorded in Gaza city as T1DM patients (38 males and 42 females) aged from 1 - 25 years. Anti-TPO, anti-TG, and anti-GAD antibodies were determined by ELISA technique and (FT4, TSH) were determined by Axsym.

Results: Anti-GAD Abs were positive in 36(45%) of patients, (61.1% male and 38.9% females). Anti-TPO Abs were positive in 44 (55%) were (40.9% males and 59.1% females). Anti-TG Abs were observed in 46 (57.5%) patients (39.1% males, and 60.9% females), TSH were high in 7 (8.7%) patients while FT4 were high in 4 (5.0%) patients. Found a high significant correlation between anti-GAD Abs and anti-TG Abs ($P= 0.001$), equivalent correlation was found between anti-GAD Abs and anti-TPO Abs ($P=0.001$) and highly significant correlation were existed between anti-GAD and both anti-(TPO, TG) Abs was significant ($P= 0.018$) where the percentage of positive anti-GAD Abs and both anti-Thyroids antibodies was 77.8%. In addition the relation between anti-TPO and anti-TG Abs also highly significant ($P=.000$).

Conclusion: strong relation was detected between T1DM and thyroid autoimmune markers which can predict the possibility to get thyroid diseases. The study concluded that anti-GAD Abs test could be used as confirmatory diagnostic test for T1DM patient, also anti-TG Abs anti-TPO Abs TSH, and FT4 could be considered as screening tests for all T1DM patients to investigate thyroid disease among them.

()

anti-GAD Abs

anti-TPO Abs anti- TG Abs

anti- GAD Abs

anti GAD Abs,

(T4 TSH)

-1)

42 38 80 (25

:

ELISA (anti-GAD Abs anti-TG Abs anti-TPO Abs)

.Axsym FT4, TSH

36 anti- GAD Abs :

(% 38.9) (% 61.1) % 45

(% 40.9) (% 55) 44 anti-TP Abs

) (% 57.5) 46 anti-TG Abs (% 59.9)

TSH (% 60.9) (%39.1

(% 5) 4 FT (% 8.7) 7

anti-TG anti-GAD Abs

(P= 0.001) Anti-TPO Abs (p = 0.001)Abs

(anti- TG Abs anti-TPO Abs) anti- GAD Abs

Anti- GAD Abs (p= 0.018)

anti- TG anti-TPO Abs % 77.8

. Abs

anti-GAD Abs

TSH, :

FT4, anti- TPO Abs anti-TG Abs

List of abbreviation

| | |
|----------------|--|
| ADA | American Diabetes association |
| AITD | Autoimmune thyroid disease |
| APS | Autoimmune polyendocrine syndrome |
| ATA | American thyroid association |
| CD | Celiac disease |
| DM | Diabetes Mellitus |
| T1DM | Diabetes Mellitus Type 1 |
| EIA | Enzyme immunoassay |
| FT4 | Free thyroxin |
| GAD-Abs | Glutamic acid decarboxylase antibody |
| IAA | Insulin autoantibodies |
| ICA | Islet-cell antibodies |
| ICSA | Islet-cell surface antibodies |
| IDDM | Insulin dependent Diabetes Mellitus |
| LADA | Latent autoimmune diabetes of adulthood |
| MEIA | Microparticle Enzyme Immunoassay |
| MOH | Ministry of Health |
| NIDDM | Non Insulin dependent Diabetes Mellitus |
| RIA | Radio Immunoassay |
| TA A | Thyroid Auto Antibody |
| TG-Abs | Thyroglobulin antibody |
| TPO-Abs | Thyroid peroxidase antibody |
| TR Abs | TSH receptor autoantibodies |
| TSH | Thyroid stimulating hormone |
| WHO | World Health Organization |

Acknowledgment



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I would like to express my deep thanks to all people who were involved in helping me to undertake my study, and whom without there cooperation this study would not have been possible.

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Chapter1

Introduction

1.1Diabetes mellitus

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism due to defects in insulin secretion, insulin action, or both (**World Health Organization, WHO, 1999**). DM is around 10% of population In Palestine, and the same ratio existed in Gaza strip (**Ministry Of Health, MOH, 2002**). The effects of DM include continuing damage, dysfunction and failure of various organs. DM may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. In its most severe forms, may develop and lead to coma and, in Absence of successful treatment, death. Hyperglycemia causes pathological and functional changes if takes long time before the diagnosis is made. DM long-term effects include progressive development complications of retinopathy, nephropathy, and/or neuropathy with risk of foot ulcers, and sexual dysfunction. People with diabetes are at risk of cardiovascular and peripheral vascular diseases (**WHO, 1998**).

The two major types of diabetes are type 1 (insulin-dependent DM, IDDM) and type 2 (non insulin-dependent DM, NIDDM) (**The Expert Committee, 1997**).

In type 1 diabetes, the pancreas fails to produce the insulin which is essential for survival, the disease process becomes more severe in which the immune system improperly recognizes the insulin-producing beta cells in the pancreas as foreign body and destroys them. This form develops most frequently in children and adolescents, but is being increasingly noted later in life. These diabetics must always take insulin injections (**Jennifer et al., 2004**).

Type 2 diabetes is the most common form of diabetes. The disease mechanisms in DM type 2 are not completely known, but some experts suggest that the first stage in DM type 2 is the condition called insulin resistance. Although insulin can attach normally to its receptors on liver and muscle cells, certain mechanisms prevent insulin from moving glucose (blood glucose) into these cells where it can be used. This condition leads to producing variable, even normal or high, amounts of insulin (**Larsson et al., 1998**). In the beginning, this amount is usually sufficient to overcome such resistance. Over time, the pancreas becomes unable to produce enough insulin to overcome resistance. So there is usually an abnormal rise in blood glucose right after a meal.

The tests which performed in Gaza for DM patients are fasting blood glucose, post prandial blood glucose, HbA1c, Insulin, and C-Peptide which used for diagnosis and prognosis (**MOH, 2003**). A selective test will be added in our study is anti-GAD Abs.

Type 1 diabetes is a typical organ-specific autoimmune disease where insulin-producing beta cells are destroyed by immune mediated mechanisms, and it can be diagnosed by detecting specific antibodies beside other clinical symptoms. The first evidence that T1DM might be an autoimmune disease origin came from the observation that T1DM is often associated with other endocrine autoimmune disorders (**Imagawa et al., 1999**).

This overlap of different autoimmune disorders has led to the concept of autoimmune polyendocrine syndrome (APS) with the clinical or sub-clinical involvement of several organs in the same subject or family, which is supposed to be the result of an interchange among genetic, hormonal, and immunological factors. Circulating auto-antibodies are a hallmark of clinical or sub-clinical autoimmune polyendocrine disease, particularly in type 1 diabetes autoimmune thyroid disease (**Jennifer et al., 2005**).

Therefore it has been postulated that the detection of thyroid antibodies, might reflect the developing of other autoimmune diseases, such as DM. This led to the suggestion that the search for thyroid antibodies could be a good prognosis for other autoimmune diseases and especially DM.

1.2 Thyroid gland

The thyroid gland function is to produce thyroid hormones which are responsible for regulating metabolism and different organs function. Disorders of the thyroid gland can be classified to hypo or hyperthyroid.

Hypothyroidism is a condition in which thyroid gland produces lower rates of thyroid hormones. The most common symptoms noticed include being very tired, hair loss, gaining weight, and constipation. In newborns, the lack of thyroid hormone results in a condition called cretinism; the child grows up mentally retarded and with growth deformities. There for all newborns in Palestine are screened for hypothyroidism to help prevent this condition. Hypothyroidism can also be acquired later in life if the body produces abnormal cells (antibodies) that can damage the thyroid gland (Hashimoto thyroiditis), which cause autoimmune hypothyroidism. Hashimoto's thyroiditis is usually accompanied by a goiter (enlargement of the thyroid gland) **(Guha, 2002)**.

Hyperthyroidism is a condition in which thyroid gland is over active and tends to produce excessive amount of the thyroid hormones. The symptoms start to appear, due to increasing body's metabolism, patients often feel hotter than those around them and can slowly loose weight even though they may be eating more **(American Thyroid Association, ATA 2002 a)**. Patients with hyperthyroidism usually have trouble sleeping. Trembling of the hands and a high or irregular heartbeat (called palpitation) may also develop. When hyperthyroidism is severe, patients can suffer shortness of breath, chest pain, and muscle weakness **(Stephanie et al., 2005)**.

Grave's disease which is classified as common hyperthyroid autoimmune disease. It is a condition caused by the patient's own immune system attack the patient's own thyroid gland. The hyperthyroidism of Grave's disease, therefore, is caused by antibodies that the patient's immune system makes which attach to specific activating sites on thyroid gland which in turn causing the thyroid to make more hormones **(Jenkins et al 2002)**.

1.3 Objectives

1.3.1 General objective

To evaluate thyroid and glutamic acid decarboxylase Autoantibodies (anti-TG, anti-TPO, and anti-GAD Abs) status in type 1 diabetes mellitus patients in Gaza, Gaza strip.

1.3.2 Specific objectives

- To evaluate anti-GAD Abs as markers of autoimmune T1DM disease.
- To evaluate anti-TG, and anti-TPO Abs as markers of autoimmune thyroid disease in T1DM patients.
- To assess thyroid function as markers of thyroid disease in positive and negative thyroid antibodies in all patients of T1DM.
- To identify the relationship between autoimmune thyroid disease and T1DM.
- To identify new early detection tests for patients' diagnosis, prognosis and management.

1.4 Importance of study

- T1DM in our community constitutes around 10% of population **(MOH 2003)**.
- Focus of concern will facilitate a better diagnosis and intervention to improve health care of T1DM.
- Surveying and investigation the opportunity tests of autoimmune that recommended for improvement and effectiveness.

Chapter 2

Literature Review

2.1 Diabetes mellitus

2.1.1 Background of diabetes mellitus

The term diabetes mellitus describes a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced, or the inability of cells to use insulin properly and efficiently leading to hyperglycemia and diabetes **(WHO 2002 a)**. Insulin is a hormone that is produced by specialized cells (beta cells) of the pancreas. (The pancreas is a deep-seated organ in the abdomen located behind the stomach.) In addition to helping glucose enter the cells, insulin is also important in tightly regulating the level of glucose in the blood. After a meal, the blood glucose level rises. In response to the increased glucose level, the pancreas normally releases more insulin into the bloodstream to help glucose enter the cells and lower blood glucose levels after a meal. When the blood glucose levels are lowered, the insulin release from the pancreas is turned down **(WHO 2002 b)**

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β -cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action **(American Diabetes Association, ADA, 2004)**. Many organs in the body are then damaged or destroyed like liver, pancreas, retina, sexual organ, muscles, kidney, the blood vessels and nerves.

2.1.2 Prevalence

DM is an epidemic disease (**David et al., 2001**). In 2004, according to the WHO, more than 150 million people worldwide suffer from diabetes. Its incidence is increasing rapidly, and it is estimated that by the year 2025 this number will double. Diabetes is in the top 10, and perhaps the top 5, of the most significant diseases in the developed world. The number of deaths related to diabetes is considerably underestimated (**WHO 2002 b**). A more believable figure is likely to be around 4 million deaths per year related to the presence of the disorder. This is about 9% of the global total. Many of these diabetes related deaths are from cardiovascular complications (**ADA, 2004**).

2.1.3 Classification of diabetes

In 1980 WHO was published the first widely accepted classification of diabetes mellitus, and modified that classification in 1985. The classification expressed two major classes of Diabetes Mellitus and named them, IDDM or Type 1, and NIDDM or Type 2 (**WHO, 1999**).

Diabetes mellitus type 1

This type of diabetes, which accounts for 10% of those with diabetes, results from a cellular-mediated autoimmune destruction of the β -cells of the pancreas. The immune system incorrectly manufactures antibodies and inflammatory cells that are directed against and cause damage to patients' own body tissues. It is believed that the predisposition to develop these abnormal antibodies in T1DM is, in part, genetically inherited, though the details are not fully understood (**William et al., 2002**).

Exposure to certain viral infections or other environmental toxins may serve to trigger abnormal antibody responses that cause damage to the pancreas cells where insulin is made. These antibodies can be measured in the majority of patients, and may help determine which individuals are at risk for developing T1DM (**Hyoty, 2002**).

Markers of the immune destruction of the β -cell include islet cell autoantibodies, autoantibody to insulin, autoantibody to glutamic acid decarboxylase GAD, and autoantibody to the tyrosine phosphatases IA-2 and IA-2 β . One and frequently more of these autoantibodies are present in 85–90% of individuals when fasting hyperglycemia is initially detected (ADA, 2006).

In type 1 diabetes, the rate of β -cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress (ADA, 2005).

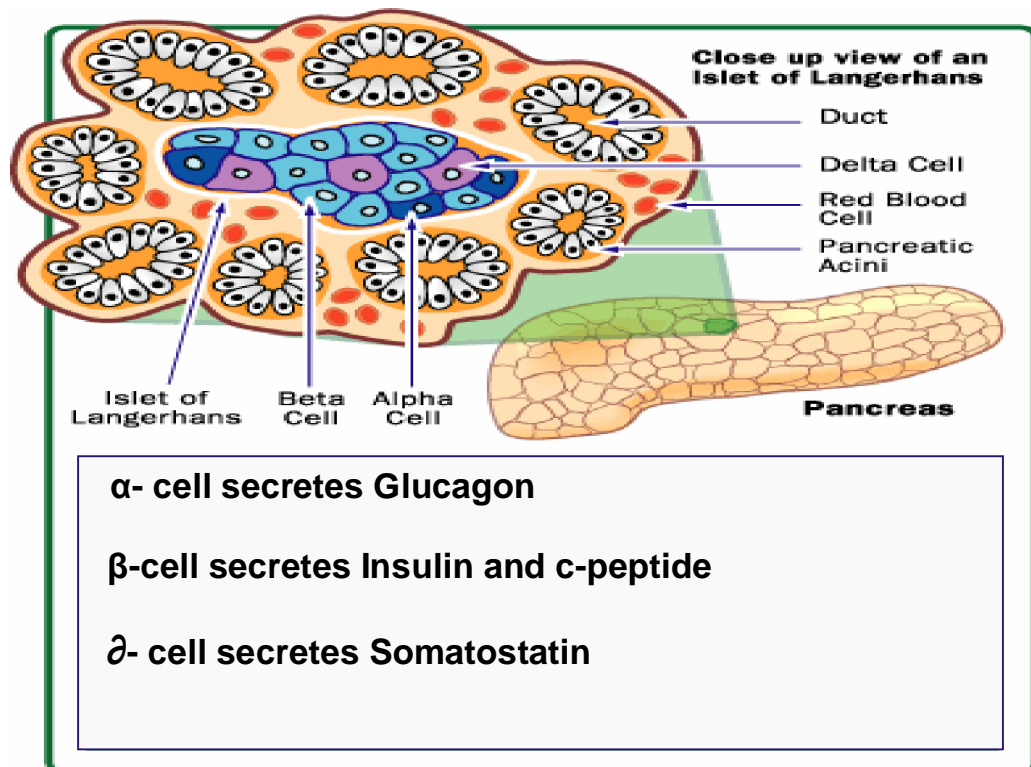


Fig 2.1 Islet cell (langerhans) Anatomy

Autoimmune destruction of β -cells has multiple genetic predispositions and is also related to environmental factors that are still poorly defined. These patients are also having a tendency to other autoimmune disorders such as Graves' disease, Hashimoto's thyroiditis, Addison's disease, celiac spur, and pernicious anemia (**Kinova et al., 1998**).

Diabetes mellitus type 2

This type of diabetes, which accounts for 90% of those with diabetes (**WHO 2002 a**), previously referred to as non-insulin-dependent diabetes, or adult-onset diabetes which always diagnosed in patients >30 years, encompasses individuals who have insulin resistance and usually have relative insulin deficiency. Although the specific etiologies are not known (**WHO, 2006**), autoimmune destruction of β -cells does not occur. Most patients with this form of diabetes are obese, and obesity itself causes some degree of insulin resistance. It usually arises in association with the stress of another illness such as infection. This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes, whereas patients with this form of diabetes may have insulin levels that appear normal or elevated. Thus insulin secretion is defective in these patients and insufficient to compensate for insulin resistance. While it is said that type 2 diabetes occurs mostly in individuals over 30 years old and the incidence increases with age, the risk of developing this form of diabetes increases with age, obesity, and lack of physical activity (**Jennifer, 1999**).

2.1.4 Diabetes mellitus symptoms

All the symptoms of untreated diabetes are related to elevated blood sugar levels, and loss of glucose in urine. High amounts of glucose in urine can cause increased urine output and lead to dehydration. Dehydration causes increased thirst and water consumption. The inability to utilize glucose energy eventually leads to weight loss despite an increase in appetite. Some untreated diabetes patients suffering complain of fatigue,

nausea vomiting and blurred vision. Elevated glucose levels can also lead to lethargy and diabetic coma (**The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus, 2003**).

2.1.5 Diabetes mellitus diagnosis

The diagnosis of DM is normally based on high levels of glucose, however it should not be based on a single glucose determination but requires confirmatory symptoms and testing strategies. Normal fasting plasma glucose levels are less than 100 (mg/dl). Fasting plasma glucose levels of more than 126 mg/dl on two or more tests on different days indicate diabetes. A random blood glucose test can also be used to diagnose diabetes. Random blood samples may be used to test for diabetes when symptoms are present. A blood glucose level of 200 mg/dl or higher indicates diabetes (**WHO 1999 and 2006**).

2.1.6 Autoantibodies as markers of autoimmune DM

T1DM is an autoimmune disease, as pancreatic β cells are distracted. Autoimmune-mediated inflammatory processes may be a transient phenomena occurring before, during and after the diagnosis of T1DM (**Robert et al., 2000**).

The majority of the β cells of the pancreatic islets are destroyed at the time of clinical diagnosis. The loss of β cells is associated with multiple immunologic phenomena, best reflected by the appearance of autoantibodies to one or more of the following islet autoantigens: a 65-glutamic acid decarboxylase GAD, an insulinoma antigen-2 (IA-2 or ICA512), or insulin itself (**Kukreja et al., 1999**). Since in autoimmune diseases the immune response is itself part of the disease process, also antibodies may reflect the presence, nature, and intensity of the immune response, it is possible to use autoantibodies as markers of disease activity. Therefore the presence of autoantibodies to all three antigens is strongly predicts type 1 diabetes in first-degree relatives of individuals with the disease. The appearance of GAD autoantibodies in

patients classified with type 2 diabetes seems to be the best predictor for the progression to type 1 diabetes (**Lethagen et al., 2002**).

Glutamic acid decarboxylase (GAD)

Glutamic acid decarboxylase (GAD; glutamate decarboxylase, L-glutamate 1-carboxy-lyase), which catalyzes formation of gamma-aminobutyric acid from L-glutamic acid, is detectable in different isoforms with distinct electrophoretic and kinetic characteristics. The enzyme has also been concerned as an auto-antigen in the autoimmune disease stiff man syndrome as it's in T1DM.

GAD is neither β -cell nor islet specific. GAD is expressed predominantly in the nervous system. Other tissues that express GAD include the testes, ovary, adrenal, pituitary, thyroid, and kidney (**Feeney et al., 1997**)

Because GAD autoantibodies are more persistent than ICA Abs after the diagnosis of T1DM (**Lohmann et al., 2001**), GAD Abs may be more often positive than ICA Abs in LADA. Because the frequency of LADA is 5–15% based on ICA Abs studies, using GAD Abs as the autoimmune marker, LADA prevalence in phenotypic type 2 diabetes might be even greater. GAD Abs has been a major focus of diabetes research for more than 10 years. GAD Abs is detected in 60% or more of new-onset cases of T1DM and 3–5% of relatives (**William et al., 2002**).

2.2 Existence of anti-GAD Abs in T1DM

Autoimmunity directed against pancreatic islet cells results in slowly progressing. The autoimmune phenomena associated with the disease include lymphocytic infiltration of the islets and circulating serum antibodies to various islet-specific antigens (**Richter et al., 1992**). So T1DM is shared with serum antibodies that precipitate a 64-kDa pancreatic islet cell protein reported to be glutamic acid decarboxylase (**Aizpurua et al., 1992**) and found that GAD is an autoantigen in a majority of T1DM patients. The lower rate of GAD antibodies in recent-onset T1DM subjects indicates either that

immunoreactivity is lost with near-total beta-cell destruction or that GAD antibodies denote a low risk of progression to clinical disease.

Several β -cell antibodies are detectable at diagnosis of diabetes: ICA, GAD, and IAA. **Maugendre et al., (1997)** established that combined markers of anti-pancreatic autoimmune response ICA, GAD and IAA antibodies enhanced the predictive value for diabetes. Also, **Pardini et al., (1999)** found that Brazilian T1DM have variable islet cell-specific autoantibodies such as ICA, anti-insulin, anti-glutamic acid decarboxylase antibodies and the antibody against tyrosine phosphatase like protein known as ICA-512 (IA-2). The frequency of the antibodies in recent-onset T1DM patients was 80.0% for GAD Abs, 62.9% for IA-2 Abs and 82.9% for GAD Abs and/or IA-2 Abs. The long-duration T1DM subjects presented frequencies of 54.1% for GAD Abs and IA-2 Abs, and 67.5% for GAD and/or IA-2 antibodies.

Imagawa et al., (1999) were looking for the relation between the development of T1DM and immunomarker GAD, and ICA by testing the patients for GAD Abs and ICA Abs, All patients were also followed monthly for 2 years and their fasting plasma glucose, haemoglobinA1C and daily insulin doses were recorded. The clinical course of patients with islet immunological abnormalities was compared with that of patients without those abnormalities. They found that the heterogeneous clinical course observed following diagnosis in patients with diabetes Type 1 correlates with islet immunological abnormalities. GAD Abs is highly prevalent in diabetes type 1, but their functional role in the pathogenesis of the disease and their relationship to GAD is still unclear. GAD Abs is also found in some individuals without type1 diabetes (**Helena et al., 2000**).

Desailloud et al., (2000) were determined ICA, GAD Abs and IA Abs in 61 patients with an initial diagnosis of type 2 diabetes but having at least one symptom suggesting slow type 1 diabetes. They found that both ICA and IA Abs were significantly less frequent in slow onset T1DM than in T1DM. GAD Abs was as frequent in slow onset as in diabetes type 1. They concluded that GAD and ICA Abs are useful markers to predict future T1DM. **Mathias et al., (2001)** were also found that autoantigens such as GAD and ICA can be

combined successfully in a fusion protein of similar immune reactivity in T1DM.

David et al., (2001) found that the simultaneous presence of autoantibody to the GAD, an insulinoma antigen (ICA), or insulin itself antigens strongly predicts T1DM in first-degree relatives of individuals with the disease. The appearance of GAD autoantibodies in patients classified with type 2 diabetes seems to be the best predictor for the progression to T1DM. Also GAD and IAA autoantibody detections are currently being used to identify individuals at risk for T1DM in several large screening programs involving newborns, schoolchildren, first-degree relatives, and the general population.

Elkadhi et al., (2002) looked for the prevalence of GAD antibodies and its association with ICA antibodies in Tunisian T1DM children. The prevalence of GAD antibodies was 51.2% and decreased as a function of increasing duration of the disease. Their frequency was 84.6% in children with newly diagnosed diabetes (within 6 months of diagnosis) and only 29.41% in those with a longer duration of the diabetes (more than 5 years). ICA Abs was present less frequently (21.4% of the children). This means that the GAD antibodies seem to be more associated to the development of type 1 diabetes than ICA Abs. They are detected more frequently in patients with long standing disease, that's making their determination very interesting as diagnostic and predictive marker.

William et al., (2002) found that many autoantibodies can be detected at the onset of type 1 diabetes including ICA, ICSA, IAA, and GAD auto antibodies. At onset of T1DM, IA Abs occur in 35–60% of children but were decidedly less common in adults, GAD Abs were more persistent than ICA after the diagnosis of T1DM so GAD Abs is more often positive than ICA Abs.

Rodacki et al., (2004) investigated whether the duration of disease has any influence on the prevalence of GAD Abs in Brazilian patients with T1DM and variable disease duration. A total of 83 patients with T1DM have been chosen to the study for GAD Abs measurement. Four groups of

patients were established according to disease duration: A) 1-5 years of disease (N = 24), B) 6-10 years of disease (N = 19), C) 11-15 years of disease (N = 25), and D) >15 years of disease (N = 15). GAD Abs prevalence and its titers were determined in each group. GADA Abs was positive in 38 patients (45.8%) and its frequency did not differ between the groups. The prevalence was in A group 11/24 (45.8%), 8/19 (42.1%) in B, 13/25 (52%) in C, and 6/15 (40%) in D group. Sex, age at diagnosis or ethnic background had no significant effect on GAD Abs (+) frequency. Duration of disease did not affect significantly the prevalence of GAD Abs or its titers in patients with T1DM after one year of diagnosis

Prazny et al., (2005) in their study on T1DM patients for specific antibodies to GAD, and IAA, they found that Anti-GAD Abs is positive in 55% of males and positive in 69% of females and Anti-IA2 α Abs is positive in 18% of males and positive in 9% of females.

Sarah et al., (2005) have been showed that among 173 children aged 0–15 years measured at diagnosis of T1DM for ICA, and GAD Abs, 132 (76%) had positive titers for ICA and 115 (66%) had GAD Abs

2.3 Thyroid gland

2.3.1 Thyroid gland definition

The thyroid gland is a large gland situated in the front part of the neck below the Adam's apple. It takes the shape of a butterfly with two wings being represented by the left and right thyroid lobes which wrap around the trachea. The two lobes of the gland are joined together by a ridge of thyroid tissue called the isthmus. Normally, the thyroid gland cannot be seen and can barely be felt, but if it becomes enlarged (goiter) which can be noticed and feel it easily and a prominent swell may appear below or to the sides of the Adam's apple (**Bryer-Ash, 2001**). The main function of the thyroid is to make thyroid hormone. This hormone has an effect on nearly all tissues of the body.

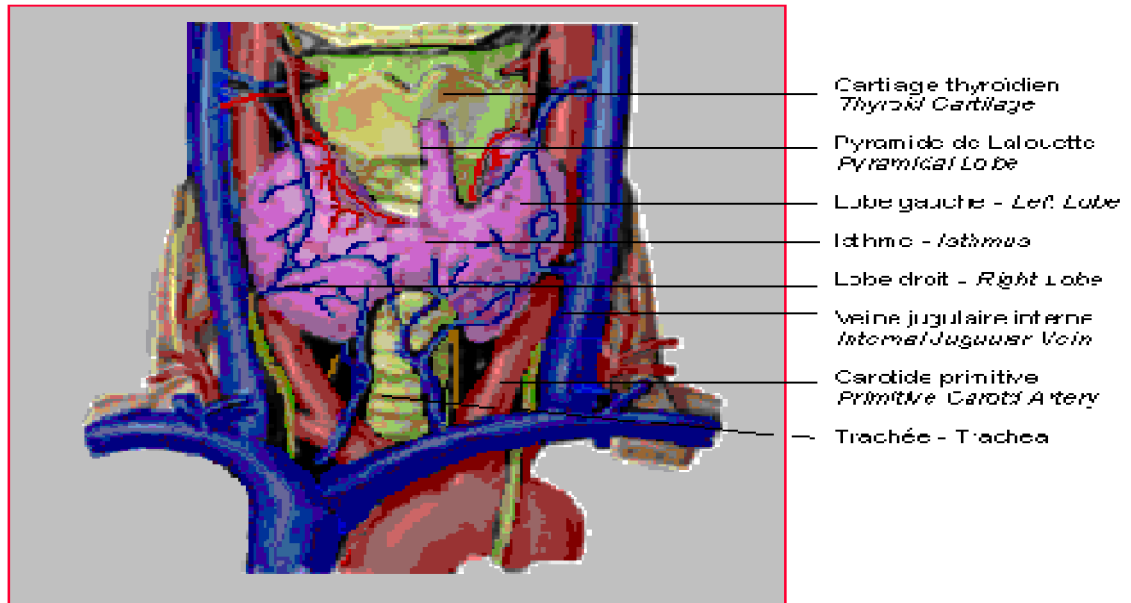


Fig 2.2 Thyroid gland anatomy

2.3.2 Thyroid hormones and their actions

The primary function of the thyroid is production and secretion of the thyroid hormones, which are chemicals produced by the thyroid to help regulate how cells work, and the organs which made up of those cells **(Brent,1994)** .

The thyroid hormones are thyroxin (also called T4 because it contains four iodine atoms) and triiodothyronine (also called T3 because it contains three iodine atoms), which are released into the blood stream. In general hormones are chemical messengers that travel from the gland that produces them directly to the body's cells and tissues **(Boelaert et al., 2005)**. When thyroid hormone reacts with a specific type of cell, for instance the cardiac cells, the cardiac muscle receive a specific order, such as the command to beat faster or more forcefully. This means that without adequate thyroid hormone, the heart's function is sluggish.

In hypothyroidism, which a condition caused by insufficient thyroid hormone the heartbeat is slow and the blood vessels move blood slowly, causing congestion **(ATA 2002 b)**. In hyperthyroidism which a condition

caused by overproduction of thyroid hormones, the heartbeat is rapid and blood pressure often rises (**Bernadette et al., 2005**). The heart, like all of the body's organs, needs adequate thyroid hormone to do its job properly. Thyroid hormones are responsible for regulating metabolism and organ function by stimulate diverse metabolic activities of most tissues, leading to an increase in basal metabolic rate, (lipids, and carbohydrate metabolism) (**Wilson, 1998**).

Also Thyroid hormones are clearly necessary for normal growth in children, as evidenced by the growth-retardation observed in thyroid deficiency. In the other hand normal levels of thyroid hormone are essential to the development of the fetal and neonatal brain.

2.3.3 Thyroid hormones synthesis and regulations

The thyroid hormones are synthesized in the follicular cells of the thyroid. The first step to hormone synthesis is the import of iodide into the follicular cells, once in the follicular cell; iodide is converted into iodine by the enzyme thyroid peroxidase (TPO). Thyroid peroxidase catalyzes the incorporation of iodide molecule onto both the 3 and/or 5 positions of the phenol rings of tyrosines found in the very large glycoprotein called thyroglobulin(TG) (**Vali et al., 2000**). Thyroid peroxidase also appears to couple iodinated tyrosine rings to convert into either T₄ or T₃. The whole thyroglobulin protein with its thyroid hormones is stored in the lumen of the thyroid follicle cell, enzymatic degradation of thyroglobulin to effect release of the thyroid hormones (**William et al., 2000**).

Thyroid hormones synthesis is regulated by the hypothalamus and the pituitary gland axis. The hypothalamus sends a signal to the pituitary gland through a hormone called TRH (thyrotropin releasing hormone), and the pituitary gland then releases TSH (thyroid stimulating hormone) to the thyroid gland. The thyroid gland then releases T₄ and T₃ hormones, which enter the bloodstream and affect the metabolism of the heart, liver, muscle and other organs. The pituitary gland regulates the level of thyroid hormone in the

blood and increases or decreases the amount of TSH released. (Shames et al., 2002)

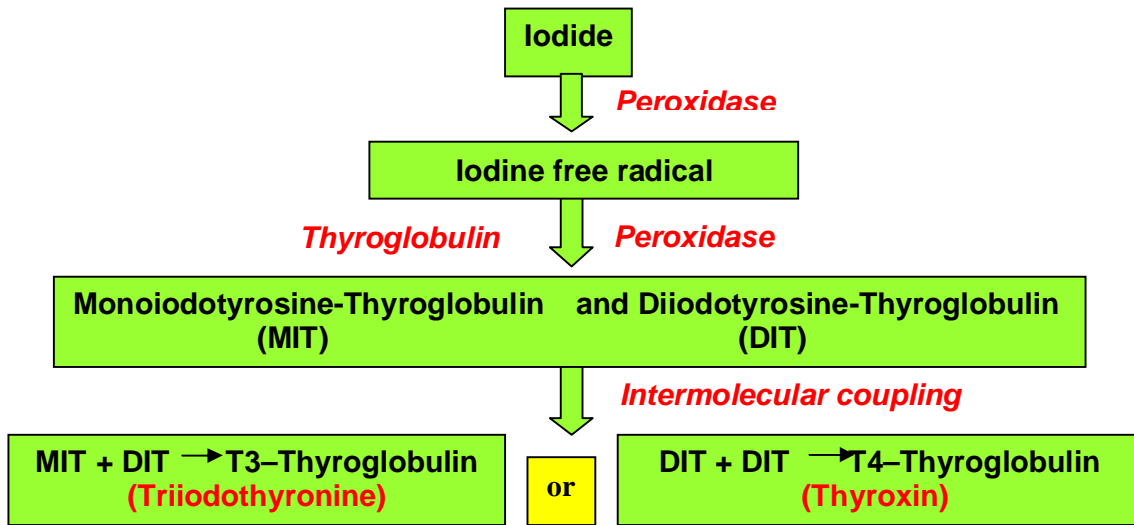


Fig 2.1 Diagram illustrates thyroid hormones production

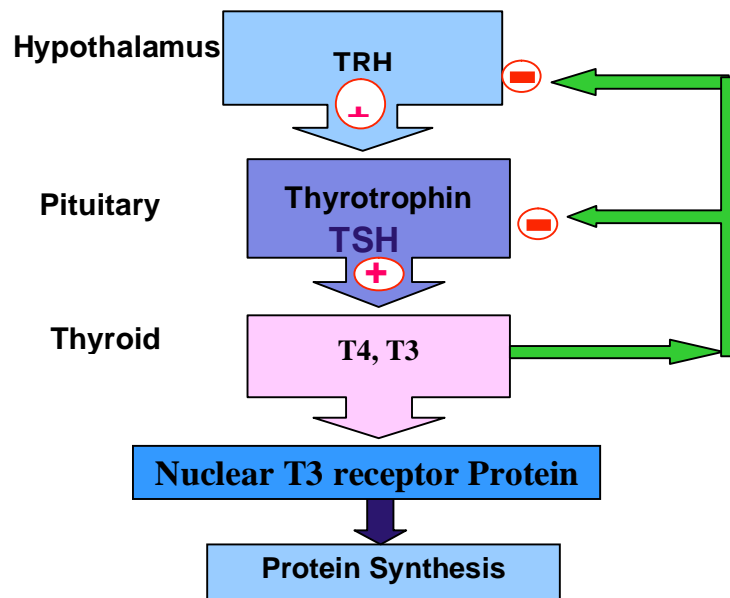


Fig 2.2 Diagram demonstrates thyroid hormones regulation and action

Negative feedback mechanism prevent the overproduction or underproduction of thyroid hormones, the pituitary gland can sense how much hormone is in the blood and adjust the production of hormones accordingly. When there is too much thyroid hormone in the blood, the TRH does not work effectively to stimulate the pituitary gland. In addition, too

much thyroid hormone will prevent the release of TSH from the pituitary gland. The sum effect of this is to decrease the amount of TSH released from the pituitary gland, resulting in less production of thyroid hormones in the thyroid gland (**Brent, 1994**).

2.3.4 Thyroid diseases states

Diseases of the thyroid gland can result in either production of too much (hyperthyroidism) or too little (hypothyroidism) hormone.

Hypothyroidism

Hypothyroidism is almost due to disease within thyroid gland that causes a decrease in the production of thyroid hormone. The most common cause of this disorder is autoimmune thyroid disease. There is a genetic predisposition to autoimmune thyroiditis although the mode of inheritance has not yet been defined (**Hueston, 2001**). Several environmental factors can trigger the onset of the disease. The other is an idiopathic thyroid degeneration and atrophy. Hypothyroidism is a common disease which slowly progressive, typically occurring in older ages anyway not until 2 years of age, women are more predisposed than men (**Michael et al., 2001**). Hypothyroidism during fetal development or early infancy results in cretinism (congenital hypothyroidism) which causes respiratory difficulties, persistent jaundice, and hoarse crying, stunted growth (dwarfism), bone and muscle dystrophy, and mental deficiency in older children, and incidence 3 times more in girls than in boys. Infants not treated within first 3 months or children within two years suffer irreversible mental retardation (**Stagnaro-Green, 2000**).

Signs and Symptoms of hypothyroidism

The signs of hypothyroidism are depending on the organ which affected. As thyroid begins to fail, slight enlargement of thyroid gland (goiter), appearing as a lump or swelling, then the patient may begin to feel tired. Skin, hair, and fingernails also grow more slowly, they become thickened,

dry, and brittle. Some hair loss may be noticed. Then, as hypothyroidism becomes more severe, changes may occur in the tissues beneath skin that lead to a characteristic puffy, swollen appearance known as myxedema. This is often particularly apparent around face and eyes **(Zulewski et al., 1997)**.

Circulation is affected and heart rate slows. Since intestinal activity slows down, patient may become constipated. A few pounds of weight gain may occur. Muscles may become painful with leg cramps. Nervous system may be affected in several ways. Some memory loss may noticed, decreased ability to think, and depression. Some patients loss of balance and difficulty in walking.

In women, changes in reproductive system may cause longer, heavier, and more frequent menstruation. Their ovaries may stop producing an egg each month, and, if so, it may be difficult to get pregnant. So when you become hypothyroid, many of the affected bodily functions simply slow down **(Wilson 1998)**.

Diagnosis of hypothyroidism

In addition to a complete medical history and medical examination, diagnostic procedures for hypothyroidism may include many laboratory measurements tests.

T4 level alone is not reliable as T4 fluctuates throughout the day and there are two distinct fractions of T4 in the blood. Free T4 and T4 bound to protein. The bound form is not possible to measure. T4 level can be determined by a blood test.

TSH level is a good concomitant factor to be measured. It is not reliable to measure alone but in a connection with free T4 measurement it increases the reliability of the test near to perfection **(Wilson, 1998)**.

Hyperthyroidism

Hyperthyroidism is present when the thyroid is producing too much thyroid hormone. In the early stages of this disorder, a person may have virtually no symptoms but laboratory tests may show a suppressed (below normal) TSH. Thyroid stimulating hormone is the most sensitive test in diagnosing thyroid disorders. Every year, 350,000 people develop some kind of hyperthyroidism, and it is eight to ten times more common in women than men **(ATA, 2002 d)**.

The most common cause of hyperthyroidism is Graves' disease, also called diffuse toxic goiter. This is an autoimmune disease in which immune system over-stimulates whole gland to make too much hormone **(Institute of Endocrinology, 2005)**. About 5% of patients with Graves' disease also have some involvement with their eyes in which the eyes may become inflamed and appear enlarged. This is described as thyroid eye disease or "exophthalmos". As the hyperthyroidism becomes more severe, the symptoms may be present evidently. Hyperthyroidism is also caused by toxic nodular goiter, a condition in which one or more nodules of the thyroid becomes overactive. The overactive nodules actually act as benign thyroid tumors. Symptoms of toxic nodular goiter do not include bulging eyes or skin problems, as in Graves' disease. The cause of toxic nodular goiter is not known **(Boelaert et al., 2005)**.

Thyroiditis is other type of hyperthyroidism causes temporary hyperthyroidism, usually followed with hypothyroidism. Thyroiditis is an inflammation of the thyroid gland.

Signs and symptoms of hyperthyroidism

The signs of hyperthyroidism include: muscle weakness especially upper arms and thighs, shaking hands, speeding up of heartbeat from a normal rate of 70 or 80 to well over 100 beats per minute, and diarrhea is exist **(Hoogendoorn et al., 2004)**.

In woman, menstrual cycle may change, flow may become much lighter and the interval between menstrual periods may increase. More rarely, periods may become irregular, or may cease entirely, making it more difficult to become pregnant. If pregnancy does occur, there appears to be an increased likelihood that will have a miscarriage. In a man, breasts may become slightly larger. Eye disease "exophthalmos" is one problem that occurs only in the type of hyperthyroidism that is caused by Graves' disease. Another condition unique to Graves' disease is a very rare **(Bernadette et al., 2005)**.

Diagnosis of hyperthyroidism

The diagnosis of hyperthyroidism of any type includes a test for thyroid stimulating hormone (TSH) from the pituitary gland, which will be low, its manufacture and release turned off by high thyroid hormone levels. Thyroid hormone levels of thyroxin (T4) and triiodothyronine (T3) are increased and indicate the degree of hyperthyroidism. A radioactive scan or ultrasound may be needed to determine whether overactive thyroid nodules are the cause of the hyperthyroidism **(ATA, 2002 b)**.

2.3.5 Diagnostic tests

To determine how well the thyroid gland is functioning, several tests are used. Usually the first and best test of thyroid function is measurement of the level of TSH in the blood. However, in rare cases in which the pituitary gland is not functioning normally, the level of TSH does not accurately reflect thyroid gland function.

Thyroid hormones level in the blood (total and free), can also be measured. In some cases, the level of a protein called thyroxin-binding globulin is measured as well because it binds the thyroid hormones in the blood. Abnormal levels of this protein can lead to misinterpretation of a person's total thyroid hormone levels but will not affect the levels of unbound or free hormones in the blood, which are the effective forms.

Also an ultrasound scan can be used to measure the size of the gland, on the other hand a thyroid scan uses radioactive iodine to produce a picture of the thyroid gland that will show any physical abnormalities **(Wilson, 1998)**.

Additional testing may be necessary in Graves' and other types of autoimmune thyroid disorder include anti-TPO Abs and anti-TG Abs tests

2.4 Thyroid autoimmunity

2.4.1 Autoantibodies as markers of thyroid autoimmune diseases

Thyroid autoantibodies can reflect disease activity and progression and can be valuable in disease prediction and classification (**Rapoport et al., 2001**). There are two major clinical diseases associated with thyroid autoimmunity, hypothyroidism (Hashimoto thyroiditis) and hyperthyroidism (Graves's disease). Two of the principal thyroid autoantigens in the former are thyroid peroxidase and thyroglobulin.

Autoimmune thyroid disease (AITD) is the most common organ specific autoimmune disorder usually resulting in hyperfunction, hypofunction or both of the thyroid gland. The syndromes comprising autoimmune thyroid disease are many intimately related illnesses: Graves' disease with goiter, hyperthyroidism, Hashimoto's thyroiditis with goitre and euthyroidism or hypothyroidism. In some patients, other organ specific and non organ specific autoimmune syndromes are associated with autoimmune thyroid disease, including T1DM (**Jenkins et al., 2002**).

AITD causes cellular damage and alters thyroid gland function. Cellular damage occurs when sensitized T-lymphocytes and/or autoantibodies bind to thyroid cell membranes causing cell lysis and inflammatory reactions. Alterations in thyroid gland function result from the action of stimulating or blocking autoantibodies on cell membrane receptors. Three principal thyroid autoantigens are involved in AITD. These are thyroid peroxidase (TPO), thyroglobulin (TG) and the TSH receptor (**Liebert, 2003**).

TPO Abs appears involved in the tissue destructive processes. Thyroid peroxidase (TPO) is the primary enzyme involved in thyroid, was initially identified in 1959 as the 'thyroid microsomal antigen. TPO-Abs are the

hallmark of AITD and are present in almost all patients with Hashimoto's thyroiditis, in two-thirds of patients with postpartum thyroiditis and also in 75% of patients with Graves' hyperthyroidism. The appearance of TPO Abs usually precedes the development of thyroid dysfunction **(DeGroot et al., 1989)**.

In iodide deficient areas, serum TG Abs measurements may be useful for detecting autoimmune thyroid disease in patients with a nodular goiter and for monitoring iodide therapy for endemic goiter **(Liebert, 2003)**. The antibodies are mainly produced by lymphocytic infiltrate in the thyroid gland and only to a small extent by regional lymph nodes or the bone marrow. Unlike antibodies against TG, TPO antibodies are may be cytotoxic to the thyroid **(Joseph et al., 2002)**.

Antibodies to TSH-R mimic the function of TSH, and cause disease by binding to the TSH-R and stimulating (or inhibiting) thyroid cells. Patients with AITD may have both stimulating and blocking antibodies in their sera. The clinical picture being the result of the relative potency of each variety; blocking antibodies seem to be more common in Graves' patients with ophthalmopathy compared to those without this complication **(Trbojevic et al., 2005)**.

2.4.2 Coexistence of anti (TPO, and TG) Abs in AITD

Three principal thyroid autoantigens are involved in AITD. These are TPO, TG and the TSH receptor. **(David et al., 2001)**

TG, TPO autoantibodies that bind simultaneously to TG and TPO are present in the serum of patients with AITD and have been found to differ from monospecific TG and TPO Abs. **Estienne, (1999)** search to obtain the prevalence of TAA, anti-TG and anti-TPO Abs in a large population. The group of patients suffering from Hashimoto's thyroiditis had TAA prevalence of 40.5%, those with Graves' disease, a prevalence of 34.6% and those with post-partum thyroiditis, 16.0%. Among the non-AITD patients with positive TAA levels, are 20.7%. They conclude that the high TAA titers are consistently associated with AITD but the reverse was not found to be true.

Hasanat et al., (2000) found that the prevalence of AITD among thyroid patients was 48.4%. Specificity of anti-TPO and anti-TG antibodies were 93% and 87%, respectively. They concluded that anti-TG and anti-TPO Abs are a useful marker for AITD detected.

Alex et al., (2001) they proved that postpartum thyroiditis is defined as a syndrome of transient or permanent thyroid dysfunction occurring in the first year after delivery and based on an autoimmune inflammation of the thyroid. Relationship between the occurrence of postpartum thyroiditis and the presence of TPO antibodies; so if a pregnant women is positive for TPO antibodies early in pregnancy, her chances of developing postpartum thyroiditis are 30–52%. It is the combination of genetic susceptibility and environmental factors that lead to thyroid autoimmunity in general and also to postpartum thyroiditis. However **Kokandi et al., (2003)** said that postpartum thyroid dysfunction develops during the first 9 months in up to 50% of women who have thyroid peroxidase antibodies (anti-TPO Abs +ve).

Elizabeth et al., (2003) reported that the prevalence of high serum concentrations of thyroid antibodies varies according to race and ethnic background. In the third U.S. National Health and Nutrition Examination Survey of persons 12 years of age or older, high serum concentrations of thyroid antibodies were present in 14.3 % of whites, in 10.9 % of Mexican Americans, and in only 5.3 percent of blacks. The majority of patients with measurable thyroid antibody concentrations have normal thyroid function. In studies in England, 10 % of postmenopausal women with high serum thyroid antibody concentrations had subclinical hypothyroidism and 0.5 % had overt hypothyroidism, although euthyroid patients with high serum thyroid antibody concentrations had progression to overt hypothyroidism at a rate of 2 to 4 % a year. In a 10-year prospective study conducted in Switzerland, high serum thyroid peroxidase antibody concentrations predicted the progression of subclinical hypothyroidism to overt hypothyroidism.

Francesco et al., (2003) have shown that the autoantibodies to TG and TPO are characteristic serum markers of thyroid autoimmunity in humans and Abs to both autoantigens are frequently present in the same

patient. But they found TG and TPO may share common Abs epitopes which accumulate evidence leading to suggest the presence of bispecific TgPO Abs in thyroid autoimmunity. TgPO Abs are reported to be detectable in most patients with high titers of TG Abs and TPO Abs but absent in patients without TG Abs.

Okosieme et al., (2003) have shown that TG is one of the thyroid autoantigens recognized in patients with AITD and found that antibodies to TG are present in the serum of patients with AITD and are also sometimes present in healthy euthyroid subjects.

2.5 Relation between T1DM and Thyroid autoimmune diseases

Type1 DM is an autoimmune disease (**David et al., 2001**). It can be associated with other autoimmune endocrine disorders as well as autoimmune impairment of non-endocrine tissue. The associated autoimmune disease may influence the control of diabetes by impairing function of the respective organ.

AITD is the most frequent autoimmune disease associated with type1 DM (**Barova et al., 2004**). The screening and diagnosis of AITD are based on the assessment of autoantibodies to anti-(TPO) and anti-(TG).

The prevalence of these autoantibodies is dependent on gender, age of patient, and age at the onset of diabetes. It also varies in different geographic regions and is known to be higher in regions with higher iodine intake (**Okosieme et al., 2003**). The assessment of thyroid stimulating hormone (TSH) allows the evaluation of the thyroid gland function. Dyslipidemia and arrhythmia are the main features frequently accompanying impaired thyroid gland function in non-diabetic subjects.

There are many studies that concerned with the subject of my research or parts of it for various ethnic and various area of the world we will discuss it as followed

2.5.1 Thyroid autoimmunity in T1DM

Radaideh et al., (2003) investigated the prevalence of thyroid function and thyroid autoimmunity in patients with T1DM. A total of 79 type 1 diabetic patients were recruited in the study, and complete thyroid function, which included FT4, FT3, and TSH were done, only 64 patients had performed thyroid autoantibodies; anti-TPO Abs and anti-TG Abs. They were compared with 127 healthy subjects matched for sex and age. In the diabetic group, 7 cases (8.9%) of thyroid dysfunction were detected, 4 of these were diagnosed as subclinical hypothyroidism, whereas the other 3 had overt hypothyroidism and were on thyroxine replacement therapy. In the control group, 6 (4.7%) subjects were diagnosed as subclinical hyperthyroidism. There was a significant difference in thyroid function variables between diabetics and controls. Among T1DM patients, 7 (9.2%) had thyroid auto antibodies, 5 with positive anti- TPO Abs only and 2 with positive anti-TPO Abs and anti-TG Abs; compared with 8 (6.3%) in the control group, 4 with positive anti-TPO Abs only and 4 with positive anti-TPO Abs and anti-TG Abs. So biochemical thyroid dysfunction and thyroid autoimmunity were evident in T1DM which were apparently euthyroid, with no significant difference between diabetics and controls.

Chang et al., (1998) investigated the prevalence of anti-TPO Abs in T1DM, and compared the effect of anti-GAD Abs on the thyroid autoimmunity in patients with T1DM in Taiwan. Two hundred and seventeen sera from 243 type 1 diabetic patients were tested. The frequency of anti-GAD Abs was 45.6%, (99 of 217) and 53 (21.8%) was positive for anti-TPO Abs. Compared with those without thyroid autoimmunity, there was a female preponderance for the type 1 diabetic patients with thyroid autoimmunity (female: male, 99:91 vs. 37:16 respectively). Among the type 1 diabetic patient with thyroid autoimmunity, anti-TPO Abs tended to occur in those of older age or with long-standing disease, their data indicated that the presence of anti-TPO Abs in 21.8% of type 1 diabetic patients confirmed the strong association of AITD and T1DM without ethnic differences. The Absence of correlation between anti-TPO Abs and anti-GAD Abs in Taiwan type 1 diabetic patients

suggested genetic heterogeneity in the role of autoimmunity of T1DM and AITD among races.

Roldan et al., (1999) examined 204 T1DM patients for autoantibodies thyroid marker and they found that the prevalence of thyroid autoimmune disorders was 17.6% and, of those, chronic autoimmune thyroiditis was the most frequent. Anti-TPO Abs was more truly with the presence of chronic autoimmune thyroiditis than anti-TG Abs. Euthyroidism was the most thyroid status of the patients with positive markers (77%), but subclinical hypothyroidism (11%), overt hypothyroidism (3%), subclinical hyperthyroidism (3%) and overt hyperthyroidism (6%) were also present. AITD were the most prevalent status affecting diabetic patients.

Holl et al., (1999) conducted their study on 2305 DM patients. Anti-TPO and TG Abs were performed in 495 patients with T1DM (234 boys, 261 girls; age at last measurement: 15.4 ± 0.3 years, duration of diabetes 7.5 ± 0.2 years). The prevalence of elevated thyroid antibodies increased dramatically with age: from 3.7% in patients less than 5 years of age up to 25.3% in the age group 15-20 years. For children older than 10 years, girls were significantly more affected than boys. In children older than 10 years, basal TSH concentrations were significantly elevated in antibody-positive patients. They concluded that thyroid autoimmunity is prevalent in children and adolescents with type 1 diabetes. Adolescent girls and young women are especially affected.

Rattarasarn et al., (2000) studied the clinical significance of thyroid autoantibodies in 50 Thai patients with T1DM and their relationship with anti-GAD Abs. Anti-TG Abs and anti-TPO Abs were positive in 9 (18%) and 15 (30%) patients, respectively. Eight patients (16%) were positive for both antibodies. Two of 16 patients who were positive for anti-TG Abs or anti-TPO Abs had a previous history of hyperthyroidism prior to diabetes onset. The frequencies of thyroid antibodies were significantly increased in females and in those who had positive anti-GAD Abs. Anti-GAD Abs were negative in all of the non-diabetic patients with autoimmune thyroid disease. So about 1/4 of Thai patients with T1DM without thyroid disease had thyroid antibodies. The

frequency of thyroid antibodies was increased in female and in anti-GAD Abs positive patients.

Jaeger et al., (2001) in Germany analyzed sera from 197 recent-onset type 1 diabetic patients at the time of diagnosis, 882 first-degree relatives, and sera of 150 healthy control subjects for prevalence and co-occurrence of the following antibodies: insulin autoantibodies; anti-IA-2 Abs and islet cell antibody; anti-GAD antibodies, anti-adrenal cortex antibodies, and anti-gastric parietal cell antibodies; anti-TG Abs and anti-TPO Abs and gliadin IgG/A and tissue-transglutaminase IgA. The overall frequency of gastric parietal cell antibodies and adrenal antibodies did not differ significantly among groups. In contrast, type 1 diabetes-associated antibodies and thyroid antibodies were significantly more frequent both in recent-onset type 1 diabetic patients and in the group of first-degree relatives. The prevalence of gliadin IgG/IgA and transglutaminase IgA was significantly higher in the group of recent-onset T1DM patients, but the difference between first-degree relatives and control subjects did not reach statistical significance.

Menon et al., (2001) studied the prevalence of autoimmune thyroid disease in Indian children with T1DM by testing the antibodies to TPO and TG. Thyroid function tests and tests of glycemic control were also performed. The study was consisted of 35 children with T1DM and 32 healthy age- and sex-matched control children. These assays were repeated after six months and one year. Anti-TPO Abs were observed in 19 (54.3%) patients compared to 3 (10%) controls, and TG Abs in 11 (31.4%) patients and none of the controls. The prevalence of these antibodies was not different in boys and girls and did not change with the duration of diabetes. All patients who were positive for anti-TG Abs were also positive for anti-TPO Abs. Thyroid function tests were abnormal in one patient who was found to have Hashimoto's thyroiditis.

Christophe et al., (2001) have tested 272 T1DM patients (116 men and 156 women; mean age, 27 ± 18 yr; duration, 10 ± 9 y), 397 first degree relatives (192 men and 205 women; parents/siblings/offspring, 48/222/127; age, 22 ± 10 yr), and 100 healthy controls for anti-GAD Abs, anti-TPO Abs

and others tests. Anti-GAD Abs were present in 68% and 5%, anti-TPO Abs were present in 21% and 4.5% of diabetic patients and relatives, respectively. Thyroid antibodies and dysfunction were more prevalent in T1DM patients than in first degree relatives. The presence of these antibodies in relatives is associated with age and proband antibody status.

Kordonouri et al., (2003) investigated thyroid autoimmunity in very large nationwide cohort of children and adolescent with T1DM. They analyzed 17749 patients with T1DM aged 0.1-20 years from Germany and Austria. Anti- TG Abs and anti- TPO Abs were measured. A total of 63% patients with positive antibodies were female, compared with 45% of patients without antibodies. The prevalence of significant thyroid antibody titers increased with increasing age. So thyroid autoimmunity seems to be common in females with T1DM.

Lethagen et al., (2002) were done the study to looking for the association between GAD Abs and subclinical β -cell damage and impaired insulin secretion, they screened 441 non diabetic patients with AITD for anti-GAD Abs, and 15 (3.4%) were found positive. They matched 11 anti-GAD Abs +ve and 13 anti-GAD Abs -ve patients who were euthyroid on thyroxin supplementation, and 13 control subjects for sex, age, and body mass index and measured insulin, and C-peptide, and glucagon response to glucose and arginine. Data showed was shown that the acute insulin response to arginine was lower in anti- GAD Abs +ve than in anti-GAD Abs -ve thyroiditis subjects at glucose concentration of 14 and >25 mmol/liter. They concluded that anti-GAD Abs were associated with a decreased insulin secretion capacity in non diabetic subjects with thyroiditis, which suggests that anti-GAD Abs positivity could be a marker of subclinical insulinitis.

Kalicka-Kasperczyk et al., (2003) evaluated the prevalence of anti-TPO Abs and thyroid disorders in 219 children and adolescents with T1DM from southeast Poland aged 3.2-22.3 years. In addition to clinical assessment of all patients, determinations were made of serum anti-TPO Abs, FT4 and TSH. Positive anti-TPO Abs titer was demonstrated in 76 (34.7%) patients with type 1 diabetes. Thyroid dysfunction was detected in 11

(5.05%) patients. These 11 patients with thyroid dysfunction constituted 14.5% of the entire group of children with both T1DM and positive anti-TPO Abs titer (n=76). The present results indicate that T1DM is strongly associated with TPO Abs.

Umpierrez et al., (2003) reported that the risk of thyroid dysfunction in patients with T1DM is two- to threefold higher than in the general population. They analyzed the incidence of thyroid dysfunction over time in a cohort of 58 patients (26 men and 32 women) and prospectively followed for 18 years. Patients underwent measurement of thyroid function tests TSH, T4, and T3 every year and anti- TPO Abs at 4-year intervals. A total of 18 patients had hypothyroidism, and one patient experienced transient hyperthyroidism. The study confirms the association between autoimmune thyroid dysfunction and T1DM.

Barova et al., (2004) evaluated anti-TG Abs and anti-TPO Abs as markers of AITD in several groups of adult patients with T1DM and type 2. They were particularly interested whether the presence of thyroid antibodies is related to the positivity of anti-GAD Abs. Elevated anti-GAD Abs in 46 % (97/210) patients with T1DM was recorded. All patients with DM type 2 were anti-GAD Abs negative. At least one thyroid antibody anti-TG Abs and/or anti-TPO Abs was found in 30 % (62/210) patients with T1DM and 27 % (22/83) type 2 diabetes patients. The patients with T1DM were further grouped according to their anti-GAD Abs status. The anti-GAD Abs positive patients had a higher prevalence of anti-TG Abs than the anti-GAD Abs negative patients as well as anti-TPO Abs. At least one thyroid antibody was detected in 39% (38/97) of anti-GAD Abs positive but only in 21 % (24/113) of anti-(GAD) Abs negative patients with T1DM. They found that there was a higher frequency of thyroid-specific antibodies in anti-GAD Abs positive adult patients with T1DM than in anti-GAD Abs negative patients or in patients with DM type 2. Thyroid antibodies-positive patients have higher levels of TSH.

Kordonouri et al., (2005) investigated the incidence of autoimmune thyroiditis in pediatric patients with T1DM. Anti-TPO Abs and anti-TG Abs as well as TSH were measured in 659 patients (54.3% boys). In 126 patients,

anti-TPO and anti-TG Abs levels were followed at yearly intervals from onset up to five years of T1DM. Anti-TPO Abs above 30 U/ml and anti-TG Abs above 20 U/ml were considered positive, values above 100 U/ml as significantly raised and indicative of AITD. L-thyroxine treatment was started if TSH was higher than 4.5 μ U/ml and/or thyroid gland enlargement on thyroid ultrasound was present. At initial of study, 15.4% of patients had raised anti-TPO Abs and 14.4% anti-TG Abs. Girls had more frequently raised antibodies than boys. The cumulative incidence of AITD after 10 years of diabetes was 0.14 (0.02), being significantly higher in females (0.18 (0.03)), particularly after the age of 12 years. At T1DM onset, positive anti-TPO Abs and anti-TG Abs were present in 21 of 126 patients (16.7%). All patients with significantly increased values of anti-TPO Abs and anti-TG Abs at T1DM onset remained positive during the following five years. To early detection of AITD in children with T1DM, measurement of anti-TPO Abs and TSH at T1DM onset and in yearly intervals after the age of 12 years is recommended.

Prazny et al., (2005) carried their study on Fifty-one type 1 diabetic patients (22 men, 29 women, mean age 37 \pm 11 years, mean duration of diabetes 16 \pm 13 years). Specific antibodies to GAD, IA-2 α , and to thyroid autoantigens TPO and TG and also TSH were measured by RIA. Autoantigens of the small intestine tissue transglutaminase autoantibodies (ATTG) were evaluated by ELISA. Eleven new cases of thyreopathy (22% of patients) were detected by the assessment of anti-(TPO and TG) Abs and TSH. Two new cases of thyreotoxicosis were diagnosed during the study. Celiac disease was diagnosed in at least two cases. The screening of autoantibodies in T1DM patients could reveal subclinical cases of AITD or coeliac disease. Subclinical forms of these disorders have no influence on diabetes control. However, impaired organ function can be associated with the worsened control of diabetes as noticed on two newly diagnosed cases of thyreotoxicosis.

Sarah et al., (2005) investigated whether the presence of thyroid autoantibodies at diagnosis of type 1 diabetes in children predicts

development of thyroid. Autoantibodies included anti-TPO Abs, islet cell autoantibody, anti-GAD Abs, and insulin autoantibody were measured at diagnosis of T1DM in 173 children aged 0–15 years. The incidence for thyroid disease was 0.9 per 100 patient-years. Within 13 years, 6 of 13 children with positive anti-TPO Abs tests at diagnosis developed thyroid disease compared with 5 of 139 children with negative anti-TPO Abs tests. Five of 11 patients who developed thyroid disease had negative anti-TPO Abs tests at diagnosis. The presence of diabetes-associated autoantibodies at diagnosis anti-GAD Abs titer levels predicted pupillary abnormality. It was concluded that anti-TPO Abs at diagnosis of T1DM predict the development of thyroid.

Jennifer et al., (2005) screened 814 patients with T1DM for anti-TPO Abs, anti-TG Abs, tissue transglutaminase antibody TG Abs and 21-hydroxylase antibody (21-OHAb). Clinical disease was defined by chart review. Factors related to the presence of autoimmunity and clinical disease including age at onset of T1DM, duration of diabetes, age at screening, sex, and the presence of autoantibodies were reviewed. Results showed that the most common autoantibodies expressed were anti-TPO Abs and/or anti-TG Abs (29%), followed by TG Abs (10.1%) and 21-OHAb (1.6%). The presence of autoantibodies was associated with and predictive of disease. It was concluded that in that large group of individuals with type 1 diabetes, the expression of organ-specific autoantibodies was very high. The grouping of autoantibody expression suggests common factors contributing to the clustering.

Chapter 3

Materials and Methods

3.1 Study Design

The design of this study is a cross-sectional. It has been selected because it is useful for descriptive purposes, and data concerning more than one point were collected. Cross-sectional studies are generally carried out in a population at a point of time or over a short period; Cause and effect are being examined at the same point of time. Exposure and outcome were determined in this study by testing the collected blood samples for FT4, TSH, anti-TPO Abs, anti-TG Abs, and anti-GAD Abs.

3.2 Setting of the Study

The study was carried out in Gaza City. Sample included patients of T1DM who were been registered in central diabetology clinic in Al- Remal Health Center.

3.3 Study population

The target population of this study was T1DM patients from one to 25 years old who reside in Gaza City.

3.4 Sample size

Out of 83 patients registered as type 1diabetics in diabetic clinic in Gaza city, 80 patients(38 males and 42 females) participated in this study.

3.5 Eligibility

Inclusion criteria: All T1DM patients aged 1-25 years who were attending Al- Remal central clinic.

Exclusion criteria: All T1DM patients male and female more than 25 years, and who are not reside in Gaza City.

3.6 Sample collection and storage procedure

The preparation for the study included the following:

1- Making contact and legal approval of study with MOH.

2- Preparing agreement letter to visit the clinic and collecting samples.

Once the agreement has been offered to take all names of the patients of T1DM from central diabetology registration then the target population have been selected. Then I started calling them by telephone or visiting them to explain why I need a sample of them. Many patients were met in Al-Remal clinic at Saturday or Tuesday, days for patients to visit clinic. All patients were in fasting condition at the time of samples collection.

The time of samples collection was from 8:00 to 10:00 am in diabetology clinic, two days a week for three months (July, August and September, 2005).

Under quality control and safety procedure for sample collection, 10 ml venous blood have been collected from each patient in plain vacutainer tube by the researcher who is a well trained lab technician.

Serum was separated from whole blood for all specimens immediately using fine centrifugation. For all patients, two serum samples were sent to the lab and stored at (- 4 to -8 °c) until were tested.

3.7 Laboratory tests

One tube sample was tested for TSH, and FT4 using microparticle Enzyme Immunoassay (MEIA) techniques. The other tube sample was tested for antibodies including TPO, TG, and GAD by ELISA techniques.

3.8 Determination of tests

3.8.1 Determination of TSH

TSH quantitative determination was done using MEIA kits by using Axsym device (close system) from Abbott Company according to manufacturer instructions (Ultra sensitive TSH ref. 7B39).

Reagent contents for TSH (MEIA) kit

- 1- One bottle (14.1 ml) Anti –TSH (Goat): Alkaline phosphate conjugate in TRIS buffer with protein (bovine) stabilizers. Minimum concentration: 0.1µg/ml. Preservative: Sodium Azide. (Reagent Bottle 1)
- 2- One bottle (9.0 ml) Anti–TSH (Mouse, monoclonal) coated Microparticles in TRIS buffer with protein (bovine) stabilizers. Preservative: Sodium Azide. (Reagent Bottle 2)
- 3- One Bottle (21.5 ml) LDS wash buffer containing surfactant. (Reagent Bottle 3)
- 4- Bottle (47 ml) TRIS buffer. Preservatives: Sodium Azide and Antimicrobial Agents. (Reagent bottle 4)
- 5 - Two bottles (4 ml each) of Ultra sensitive TSH master calibrators.

| Bottle | TSH concentration (µIU/ml) |
|---------------|----------------------------|
| Master CAL. 1 | 0 |
| Master CAL. 2 | 2 |

- 6- Three bottles (8 ml each) of Ultra sensitive TSH controls.

| Bottle | TSH concentration µIU/ml | Range (µIU/ml) |
|-----------|--------------------------|----------------|
| Control L | 0.25 | 0.15 – 0.35 |
| Control M | 6.0 | 4.5 – 7.5 |
| Control H | 30 | 21.0 - 39 |

Manual procedure

- All reagents and solutions need for doing the test and operate the Axsym is placed in its special place in Axsym, which then be ready to run the test.
- Calibration for TSH was done before running the test as same as test done.
- Samples, controls and all Axsym ultrasensitive TSH reagents required for the test are pipetted by the sampling probe into various wells of a reaction vessel (RV).
- Samples, controls and Anti-TSH coated microparticles are pipetted into one well of the RV forming an antibody-antigen complex.
- The RV is immediately transferred into the processing center.

- An Aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles was transferred to the matrix cell. The microparticles bound irreversibly to the glass fiber matrix.

- The matrix cell was washed by wash buffer.
- The Anti-TSH was dispensed onto the matrix cell and bind with antibody-antigen complex.
- The matrix cell was washed to remove unbound materials.
- The substrate, 4-methylumbelliferyl Phosphate, was added to the matrix cell and the fluorescent product was measured by the MEIA optical assembly.

Calculation was done automatically by Axsym (Axsym is full automated device).

Quality control

Statistically significant number of controls should be assayed to establish the mean values and acceptable range to assure proper performance and the validity of samples results according to control range.

Expected values for TSH as in the user manual: - 0.49-4.67 μ IU/ML.

3.8.2 Determination of FT4

FT4 quantitative determination was done using MEIA kits by using Axsym device (close system) from Abbott Company according to manufacturer instructions (Free T4 ref. 7A54).

Reagent contents for FT4 (MEIA) kit

1- One bottle (15.2 ml) Solubilizer solution. TRIS buffer containing preservative: Sodium Azide. (Reagent Bottle 1)

2- One bottle (12.8 ml) alkaline phosphate conjugate in TRIS buffer with protein stabilizers. Minimum concentration: 0.4 μ g/ml. Preservative: Sodium Azide. (Reagent Bottle 2)

3-One bottle (14.4ml) Anti-FT4 (Sheep) coated microparticles in TRIS buffer with protein stabilizers. Preservative: Sodium Azide. (Reagent Bottle 3)

4- One bottle (50.2 ml) TRIS buffer. Preservatives: Sodium Azide and Antimicrobial Agents. (Reagent bottle 4)

5 - Two bottles (4 ml each) of Ultra sensitive Free T4 master calibrators

| Bottle | Free T4 concentration (ng/dl) |
|---------------|-------------------------------|
| Master CAL. 1 | 0 |
| Master CAL. 2 | 1.0 |

6- Three bottles (8 ml each) of FT4 controls.

| Bottle | Free T4 concentration (ng/dl) | Range (ng/dl) |
|-----------|-------------------------------|---------------|
| Control M | 1.2 | 0.96 – 1.44 |
| Control H | 2.0 | 1.44 – 2.88 |
| Control L | 0.7 | 0.45 – 0.95 |

Manual procedure

- All reagents and solutions need for doing the test and operate the Axsym is placed in its special place in Axsym, which then be ready to run the test.
- Calibration for FT4 was done before running the test as same as test done.
- Sample and all Axsym F T4 reagents required for one test are pipetted by the sampling probe into various wells of a reaction vessel (RV).
- Sample and Anti- F T4 coated microparticles are pipetted into one well of the RV forming an antibody-antigen complex.
- The RV was immediately transferred into the processing center.
- An Aliquot of the reaction mixture containing the antibody-antigen complex bound to the microparticles was transferred to the matrix cell. The microparticles bind irreversibly to the glass fiber matrix.
- The matrix cell was washed by wash buffer.
- The Anti- F T4 was dispensed onto the matrix cell and bind with antibody-antigen complex.
- The matrix cell was washed to remove unbound materials.
- The substrate, 4-methylumbelliferyl Phosphate, was added to the matrix cell and the fluorescent product was measured by the MEIA optical assembly.

Calculation was done automatically by Axsym.

Quality control

Statistically significant number of controls should be assayed to established the mean values and acceptable range to assure proper performance and the validity of samples results according to control range.

Expected values for FT4 as in the user manual: - 0.71- 1.85 ng/mL.

Others materials for operating Axsym device

- 1 - One bottle (10 ml) of specimen diluent's.
- 2- Four bottles (230 ml each) solution 1 containing 4-Methylumbellifery phosphate (MUP).
- 3 - Others (matrix cells, matrix cell wash, and line diluents solutions).

3.8.3 Anti- TPO antibodies Determination

Anti-TPO antibodies quantitative determination was done by using Enzyme Immunoassay (EIA) kits (Binding site), and Stat fax-2100 Awareness Technology Instrument as EIA reader according to manufacturer instructions (Binding site, Human anti TPO MK039). The sample was prepared to be used by dilute 10 μ L of each sample with 1000 μ L of sample diluent (1:100)

Reagent contents for anti- TPO antibodies (MIA) kit

TPO Coated wells: Twelve break apart 8 well strips coated with recombinant TPO and color coded red. Each plate is packed in a re-sealable foil bag containing two desiccant pouches.

Sample diluent's: Two bottles containing 50 ml of buffer for sample dilution. Colored yellow, ready to use.

Wash buffer 20x concentrate : One bottle containing 50 ml of a 20- fold concentrated buffer for washing the wells, which be ready by adding 50- ml vial content to 950 ml of distilled water.

TPO Calibrators: Five bottles each containing 1.2 ml of diluted human serum, with the following concentrations of anti-TPO autoantibody: 2700, 900, 300, 100 and 33.3 U/ml. Ready to use.

TPO Positive control: One bottle containing 1.2 ml of diluted human serum. The expected value is >100 U/ml. Ready to use.

Thyroid negative control: One bottle containing 1.2 ml of diluted human serum. The expected value is <75 U/ml. ready to use.

TPO Conjugate: One bottle containing 12 ml of purified peroxidase labeled antibody to human IgG coloured red, ready to use.

TMB substrate: One bottle containing 14 ml TMB substrate, ready to use.

Stop solution: One bottle containing 14 ml of 3 M phosphoric acid. Ready to use.

Manual procedure

1. Sample Addition

One hundred μL of each calibrate, control and diluted (1:100) sample was dispensed into the appropriate wells of the plate provided, NOTE: Samples should be added as quickly as possible to the plate to minimize assay drift, and the timer started after the addition of the last sample.

Then incubated wells at room temperature for 30 minutes.

2. Washing

The washing procedure is critical and requires special attention. An improperly washed plate will give inaccurate results, with poor precision and high background.

After incubation the plate was removed and washed wells 3 times with 250-350 μL wash buffer per well. Washing the plate either by using an automatic plate washer or manually as indicated below.

After the final automated wash, the plate was inverted and taped the wells dry on Absorbent paper.

3. Conjugate Addition

One hundred μL of conjugate was dispensed into each well, and blotted the top of the wells with tissue to remove any splashes.

4. Washing :Step 2 was repeated

5. Substrate (TMB) Addition

One hundred μL of the TMB substrate was dispensed into each well, and then blotted the top of the wells with tissue to remove any splashes.

Then incubated at room temperature in the dark for 30 minutes

6. Stopping reaction

Then dispensed 100 μL of stop solution into each well. This causes a change in colour from blue to yellow.

7. Optical density measurement

Each well was reading the optical (OD) at 450 nm on microplate reader within 30 minutes of stopping reactions.

Calculation was done automatically by microplate reader.

Quality control

Statistically significant number of controls should be assayed to establish the mean values and acceptable range to assure proper performance and the validity of samples results according to control range.

Expected value for anti- TPO as in the user manual

| Concentration | Result |
|----------------------|---------------|
| < 75 | Negative |
| 75-100 | Borderline |
| >100 | Positive |

3.8.4 Anti- TG antibodies Determination

Anti-TG antibodies quantitative determination was done by using Enzyme Immunoassay (EIA) kits (Binding site), and Stat fax-2100 Awareness Technology Instrument as EIA reader according to manufacturer instructions (Binding site, Human anti TG MK018). The sample was prepared to be used by dilute 10 μ L of each sample with 1000 μ L of sample diluents (1:100)

Reagent contents for anti- TG antibodies (MIA) KIT

TG Coated wells: Twelve break apart 8 well strips coated with purified TG and color coded blue. Each plate is packaged in a re-sealable foil bag containing two desiccant pouches.

Sample diluent's: Two bottles containing 50 ml of buffer for sample dilution, color red yellow (Ready to use).

Wash buffer 20x concentrate: 1 bottle containing 50 ml of a 20-fold concentrated buffer for washing the wells, which be ready by adding 50- ml vial content to 950 ml of distilled water.

TG Calibrators: Five bottle each containing 1.2 ml of diluted human serum, with the following concentrations of anti-TG autoantibody: 2700, 900, 300, 100 and 33.3IU/ml. Ready to use.

TG Positive control: One bottle containing 1.2 ml of diluted human serum. The expected value is >100 IU/ml. Ready to use.

Thyroid negative control: One bottle containing 1.2 ml of diluted human serum. The expected value is <75 IU/ml. ready to use.

TG Conjugate: One bottle containing 12 ml of purified peroxidase labeled antibody to human IgG colored red, ready to use.

TMB substrate: One bottle containing 14 ml TMB substrate, ready to use.

Stop solution: One bottle containing 14 ml of 3 M phosphoric acid. Ready to use.

Manual procedure

1. Sample Addition:

One hundred μL of each calibrate, control and diluted (1:100) sample was dispensed into the appropriate wells of the plate provided, NOTE: Samples should be added as quickly as possible to the plate to minimize assay defect, and the timer started after the addition of the last sample.

Then incubated wells at room temperature for 30 minutes.

2. Washing

The washing procedure is critical and requires special attention. An improperly washed plate will give inaccurate results, with poor precision and high background.

After incubation the plate was removed and washed wells 3 times with 250-350 μL wash buffer per well. Washing the plate either by using an automatic plate washer or manually as indicated below.

After the final automated wash, the plate was inverted and taped the wells dry on Absorbent paper.

3. Conjugate Addition

One hundred μL of conjugate was dispensed into each well, and blotted the top of the wells with tissue to remove any splashes.

4. Washing

Step 2 was repeated

5. Substrate (TMB) Addition

One hundred μL of the TMB substrate was dispensed into each well, and then blotted the top of the wells with tissue to remove any splashes.

Then incubated at room temperature in dark for 30 minutes.

6. stopping reaction

Then dispensed 100 μL of stop solution into each well. This causes a change in color from blue to yellow.

7. Optical density measurement

Each well was reading the optical (OD) at 450 nm on micro plate reader within 30 minutes of stopping reactions.

Calculation was done automatically by micro plate reader.

Quality control

Statistically significant number of controls should be assayed to establish the mean values and acceptable range to assure proper performance and the validity of samples results according to control range.

Expected value for anti- TG as in the user manual

| Concentration | Result |
|---------------|------------|
| < 75 | Negative |
| 75-100 | Borderline |
| >100 | Positive |

3.8.5 Anti- GAD antibodies Determination

Anti-GAD antibodies qualitative determination was done by using Enzyme Immunoassay (EIA) kits (DRG), and Stat fax-2100 Awareness technology instrument as EIA reader according to manufacturer instructions (Anti-GAD ELISA, EIA-1910).

Reagent contents for anti- GAD antibodies and its preparation

- 1- GAD- Microwell strip (12 strips)
- 2- GAD- Enzyme conjugate (2 bottles each 1.0ml),(concentrated, and can be ready to use by adding 5 ml of conjugate diluent to 1.0 ml of the enzyme conjugate and mix).
- 3- GAD-Sample diluents (1bottle 25.0 ml), (concentrated, and can be ready to use by adding 25.0 ml of sample diluents to 100.0 ml of the distilled water and mix).
- 4- GAD- Conjugate diluents (1 bottle 10.0ml).
- 5- GAD- Calibrators (3 bottles each 2.0ml) with concentration 1, 2, and 3 arbitrary units.
- 6- GAD- Negative control (1 bottle 2.0ml) with concentration <1.0 unit.
- 7- GAD- Positive control (1 bottle 2.0ml) with concentration >1.050 unit.
- 8- GAD-Substrate solution (1 bottle 15.0ml).
- 9- GAD- Wash buffer (1 bottle 20.0ml) (concentrated, and can be ready to use by adding 20.0 ml of wash buffer to 480.0 ml of the distilled water and mix).
- 10- GAD- Stop solution (1 bottle 6.0ml),(1 N Na OH)

Manual Procedure

All the reagents including serum sample were bring to room temperature (25⁰C) before starting assay (and be sure to prepare reagents).

1. The number of microwell strips needed for the test was assembled in the holder provided. The microwell strips must be snapped in place

firmly with its square side sitting on the numerical side of the holder, otherwise it will fall out and break. Wells A1 and B1 are reserved for blank.

2. One hundred μL (0.1 ml) of calibrators, positive and negative controls, and the diluted serum samples was dispensed into the appropriate microwells, except blank wells.
3. then the plate was covered with parafilm (to prevent contamination) and incubated the plate for 1 hour at room temperature ($25^{\circ}\text{C}\pm 1$)
4. After a one hour incubation, dumped the contents in the micro wells & blot the plate dry by tapping gently onto a paper towel a few times, washed each well three times with $300\mu\text{l}$ of the washing buffer solution, then the plate was blotted onto paper towel a few times at the end of each wash.
5. One hundred μl of reconstitution enzyme conjugate reagent was added to all micro wells except wells A1 & B1.
6. The plate was covered with parafilm & incubated it in dark at room temp. ($25^{\circ}\text{C}\pm 1$) for 1 hour.
7. At the end of the incubation the micro wells was washed three times as in step 4.
8. One hundred μl of conjugate was added to all micro wells.
9. Then the plate covered with parafilm and incubates in the dark for 30min. at room temp. ($25^{\circ}\text{C}\pm 1$).
10. At the end of 30 min. after substrate addition, $50\mu\text{l}$ of stopping solution was added to each well.
11. The plate reader was blanked & read the Absorbance of the plate at 405 nm

Calculation was done automatically by the plate reader.

Quality control

Statistically significant number of controls should be assayed to establish the mean values and acceptable range to assure proper performance and the validity of samples results according to control range.

Expected value for anti- GAD as in the user manual

| GAD value (arbitrary units) | Result |
|-----------------------------|------------|
| < 1.00 | Negative |
| 1.00-1.05 | borderline |
| > 1.05 | Positive |

3.8.6 Additional materials required

- 1- Distilled or deionized water.
- 2- Absorbent paper towels to blot and dry the strips after washing.
- 3- Glass/Plastic tubes for sample dilution.
- 4- Calibrated Micropipette with disposable tips, for dispensing 10 µl, 25 µl, 50 µl, 100 µl and 1000 µl.
- 5- Multichannel pipette.
- 6- Microtiter plate washer or a squeeze bottle for wash.
- 7- Microtiter plate reader with 405 and 450 nm Absorbance capability.
- 8- Axsym instrument for detected FT4 and TSH concentration.

Chapter 4

Results

4.1 Study Population

The results of the study revealed that 38(47.5%) of the population are males while 42 (52.5%) are females (Fig 4.1). The average actual age of the study population is 15.01 ± 5.64 years. The average age of the study population when the diabetes mellitus was diagnosed is 10.06 ± 5.46 years (Fig 4.2). Concerning the level of hormones related to thyroid gland function, the results showed that percentage of subjects of normal TSH is 91.3%, while 8.7% is of high levels. Moreover, the percentage of normal FT4 is 95%, while 5% is of high levels (Fig.4.3). Figure 4.4 showed that 36 (45%) of the study population subjects are of negative anti-TPO, while 44 (55%) are of positive anti-TPO Abs. Forty six (57.5 %) subjects are of positive anti-TG Abs, while 34 (42.5%) are negative. In addition it is observed that 36 (45%) subjects are of positive anti-GAD, While, 44 (55%) subjects are negative.

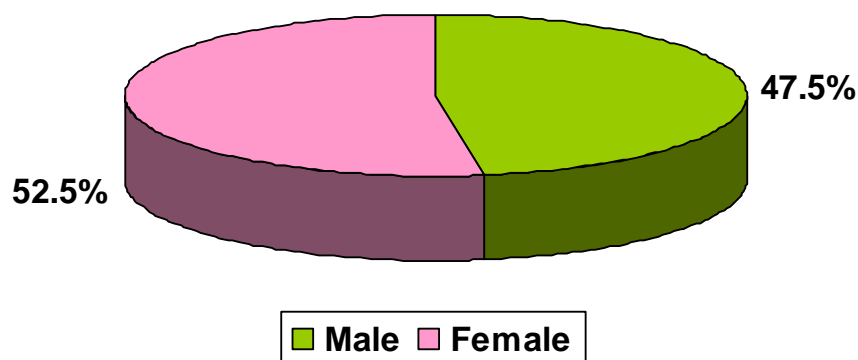


Fig. 4.1 Distribution of study population by gender

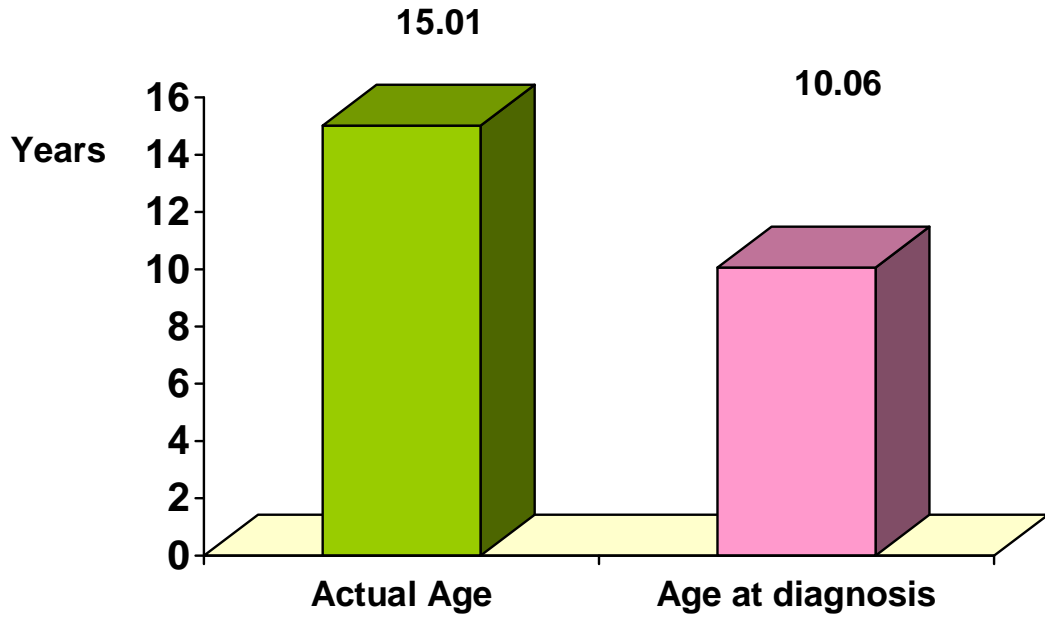


Fig. 4.2 Average age of the study population during study and at diagnosis

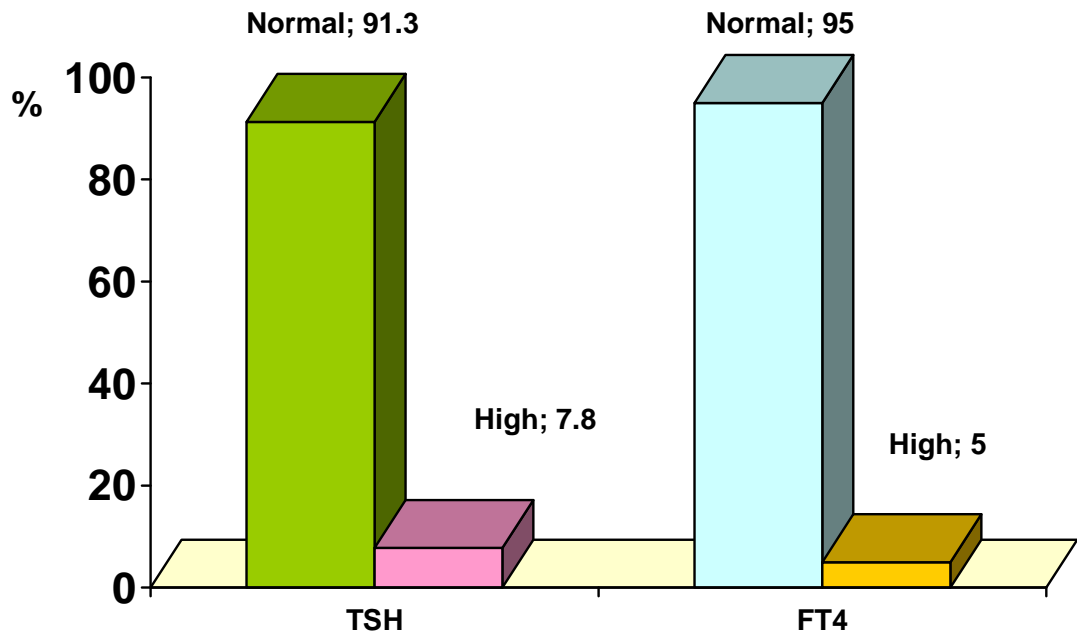


Fig.4.3 Distribution of the study population by TSH and FT4 levels

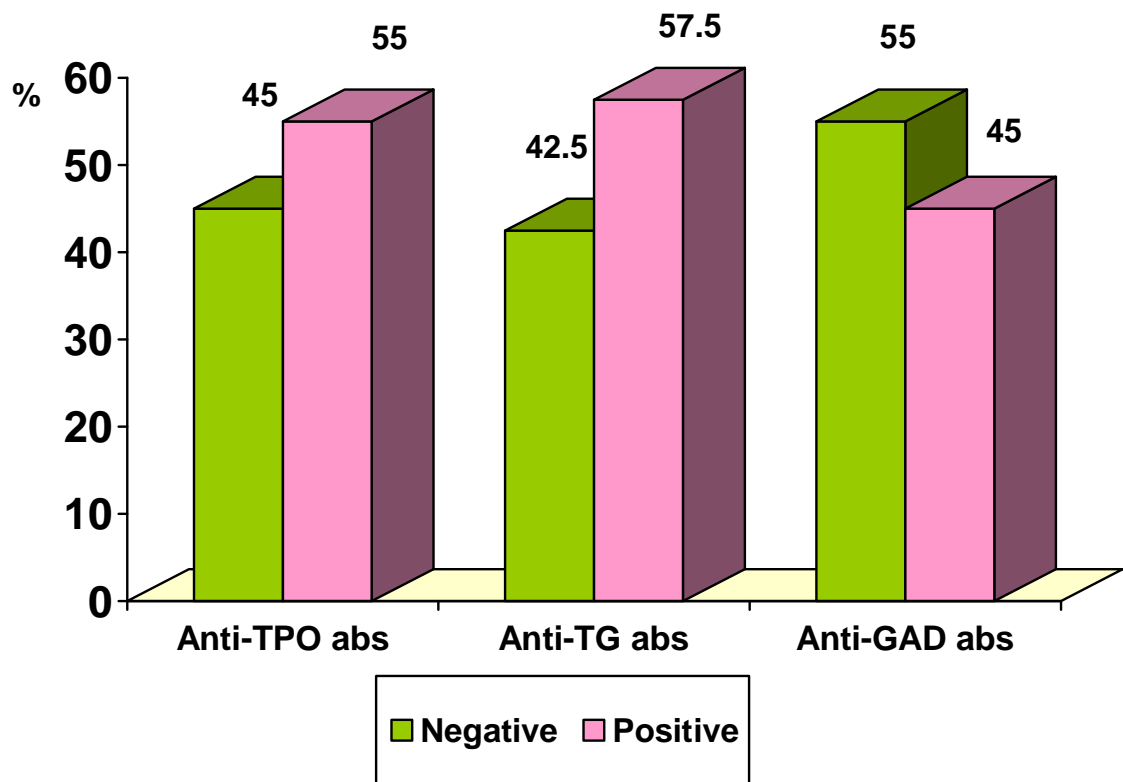


Figure 4.4 Distribution of the study population by levels of anti-(TPO,TG and GAD) Abs

4.2 Relationships of anti-GAD Abs

4.2.1 Relationship between anti-GAD Abs and Personal factors

Table 4.1 revealed a statistically significant relationship between the gender of the subjects and anti-GAD Abs ($X^2= 4.86$, $P= 0.027$), where are 61.1% of positive subjects are males and 38.9% of them are females. The negative males and females represent 36.4 and 63.6, respectively. Although there is no statistically significant differences between age groups according to the anti-GAD Abs ($X^2= 1.8$, $P= 0.41$), the percentage of positive subjects are generally increased with increasing age (Table 4.1).

Table 4.1 Relationship between anti-GAD Abs and personal variables (gender and age)

| Anti –GAD Abs Variable | Negative | | Positive | | Total | | p-value |
|---------------------------|----------|-----|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| Gender | | | | | | | |
| Male | 16 | 36. | 22 | 61.1 | 38 | 47.5 | 0.027* |
| Female | 28 | 63. | 14 | 38.9 | 42 | 52.5 | |
| Total | 44 | 100 | 36 | 100 | 80 | 100 | |
| Actual age | | | | | | | |
| -10 | 12 | 27. | 6 | 16.7 | 18 | 22.5 | 0.41 |
| 10.01- 18 | 24 | 54. | 20 | 55.6 | 44 | 55 | |
| 18.01+ | 8 | 18. | 10 | 27.8 | 18 | 22.5 | |
| Total | 44 | 100 | 36 | 100 | 80 | 100 | |

* =Significant

4.2.2 Relationship between Anti-GAD Abs and Duration of T1DM

Data results revealed a statistically significant correlation between the duration of diabetes mellitus type I and the serum level of anti-GAD Abs. So it is possible to predict the level of anti-GAD Abs (Y), by knowing the duration of T1DM (X) according to the regression equation, $[Y= 0.86+(0.28*X)]$, (P= 0.01). This is shown in (Figures 4.5 and 4.6).

4.2.3 Relationship between anti-GAD Abs and hormones related to thyroid function

Table 4.2 showed no statistically significant relationship between anti-GAD Abs and both hormones related to thyroid function (FT4 and TSH). It is important to mention that the percentage of subjects of high TSH and FT4 in this study is very low [8.7% (n= 7/80), and 5% (n=4/80), respectively].

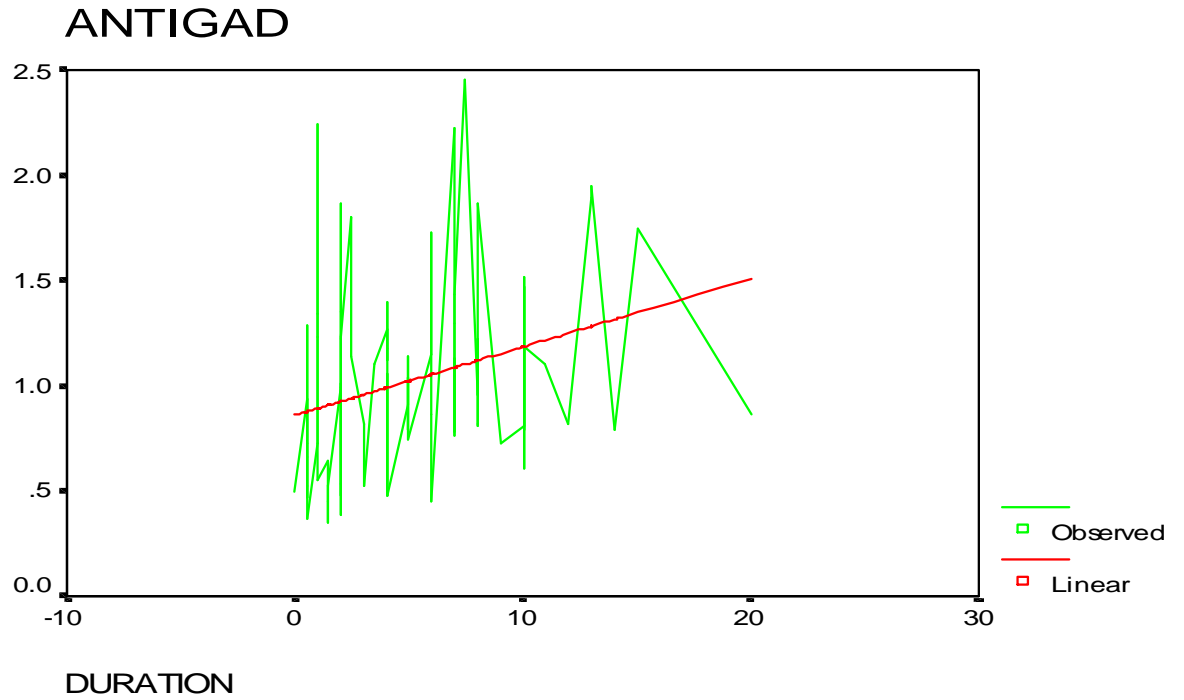


Figure 4.5 Regression line represents the prediction of the relationship between duration of T1DM and Anti-GAD Abs levels

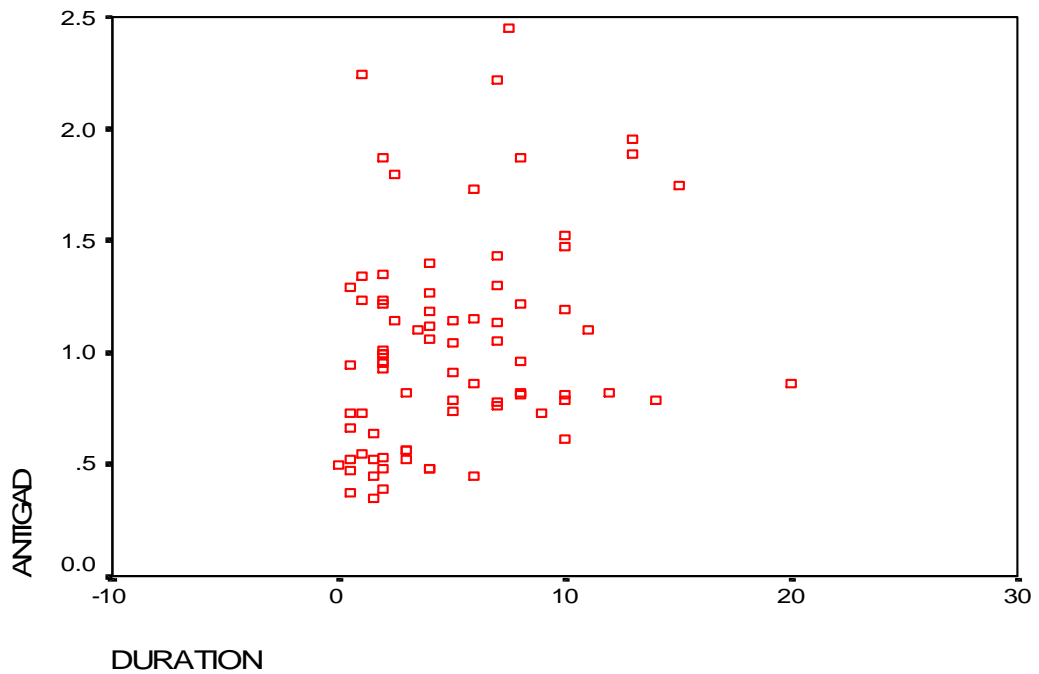


Figure 4.6 Scatter graph demonstrates the relationship between duration of T1DM and Anti-GAD Abs levels

Table 4.2 Relationship between anti-GAD Abs and hormones related to thyroid function (FT4, and TSH)

| anti -GAD Variable | Negative | | Positive | | Total | | p-value |
|-----------------------|----------|------|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| FT4 | | | | | | | |
| Normal | 42 | 95.5 | 34 | 94.4 | 76 | 95 | 0.84 |
| High | 2 | 4.5 | 2 | 5.6 | 4 | 5 | |
| Total | 44 | 100 | 36 | 100 | 80 | 100 | |
| TSH | | | | | | | |
| Normal | 40 | 90.9 | 33 | 91.7 | 73 | 91.3 | 0.91 |
| High | 4 | 9.1 | 3 | 8.3 | 7 | 8.7 | |
| Total | 44 | 100 | 36 | 100 | 80 | 100 | |

4.2.4 Relationship between anti-GAD Abs and anti-thyroid antibodies (Anti-TPO and Anti-TG)

Results showed high statistically significant correlation between anti-GAD Abs and each anti-TPO Abs, and anti-TG Abs [$\chi^2 = 10.58$, ($P = 0.001$), and $\chi^2 = 11.01$, ($P = 0.001$)], respectively. The percentage of subjects with positive anti-GAD Abs and anti-TPO Abs was 75%, while the percentage of subjects of negative anti-GAD Abs and anti-TPO Abs was 62.4%. The percentage of subjects with positive anti-GAD Abs and anti-TG Abs was 77.8%, while the percentage of negative anti-GAD Abs and Anti-TG Abs was 59.1% (Table 4.3). The equation of regression revealed that there is a correlation between anti-GAD Abs and anti-TPO Abs, and this correlation is highly significant level [$Y = 71.2 + (0.2 * X)$], ($P = 0.001$) (Figures 4.7, and 4.8). On the other hand, the equation of regression revealed high statistically significant correlation between anti-GAD Abs and Anti-TG Abs [$Y = 69.47 + (0.32 * X)$], ($P = 0.001$) as shown in Figures 4.9 and 4.10. The relation between anti-GAD Abs and both anti-TPO and anti-TG Abs was statistically significant

as shown in Table 4.3 [$\chi^2 = 5.57$, ($P = 0.018$)]. The percentage of Positive anti-GAD Abs and both anti-Thyroids was 77.8% as shown in Fig. 4.11.

Table 4.3 Relationship between anti-GAD Abs and (anti-TPO and/or anti-TG) Abs

| anti -GAD Abs Variable | Negative | | Positive | | Total | | p-value |
|------------------------------|----------|------|----------|------|-------|------|---------|
| | No | % | No | % | No | % | |
| anti-TPO Abs | | | | | | | |
| Negative | 27 | 62.4 | 9 | 25 | 36 | 45 | 0.001** |
| Positive | 17 | 38.6 | 27 | 75 | 44 | 55 | |
| Total | 44 | 100 | 36 | 100 | 80 | 100 | |
| anti-TG Abs | | | | | | | |
| Negative | 26 | 59.1 | 8 | 22.2 | 34 | 42.5 | 0.001** |
| Positive | 18 | 40.9 | 28 | 77.8 | 46 | 57.5 | |
| Total | 44 | 100 | 36 | 100 | 80 | 100 | |
| Both anti-thyroid Abs | | | | | | | |
| Negative | 21 | 47.7 | 8 | 22.2 | 29 | 36.2 | 0.018* |
| Positive | 23 | 52.3 | 28 | 77.8 | 51 | 63.8 | |

* =Significant, ** = Highly significant

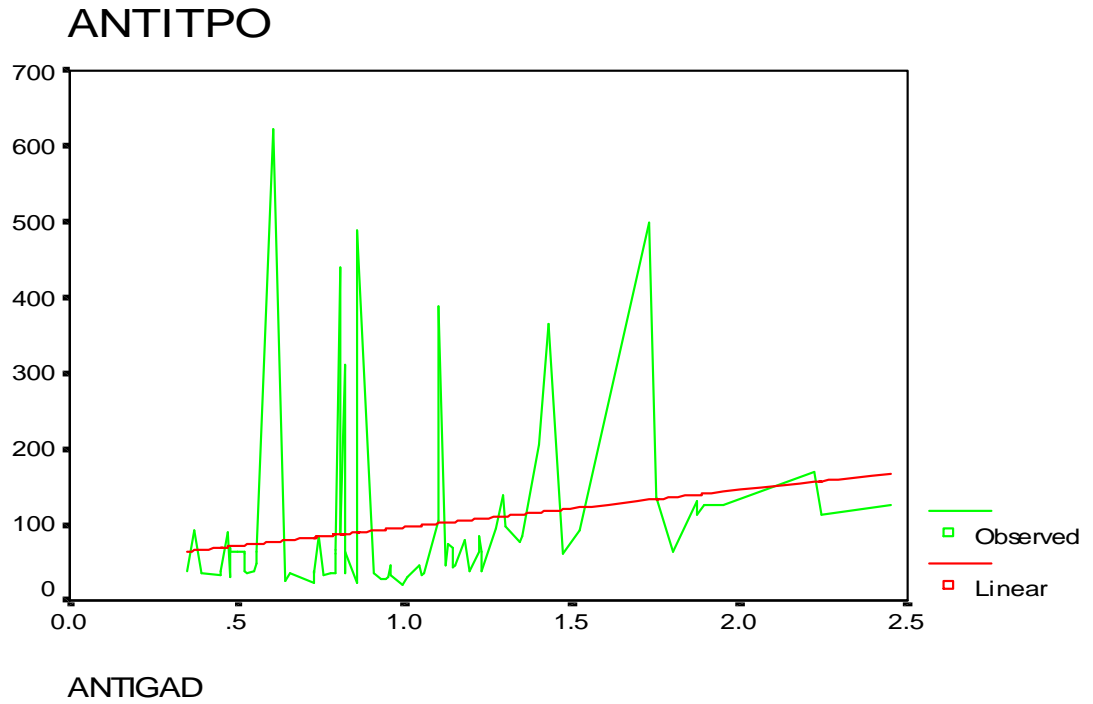


Figure 4.7 Regression line represents the prediction of relationship between anti-TPO and anti-GAD Abs levels

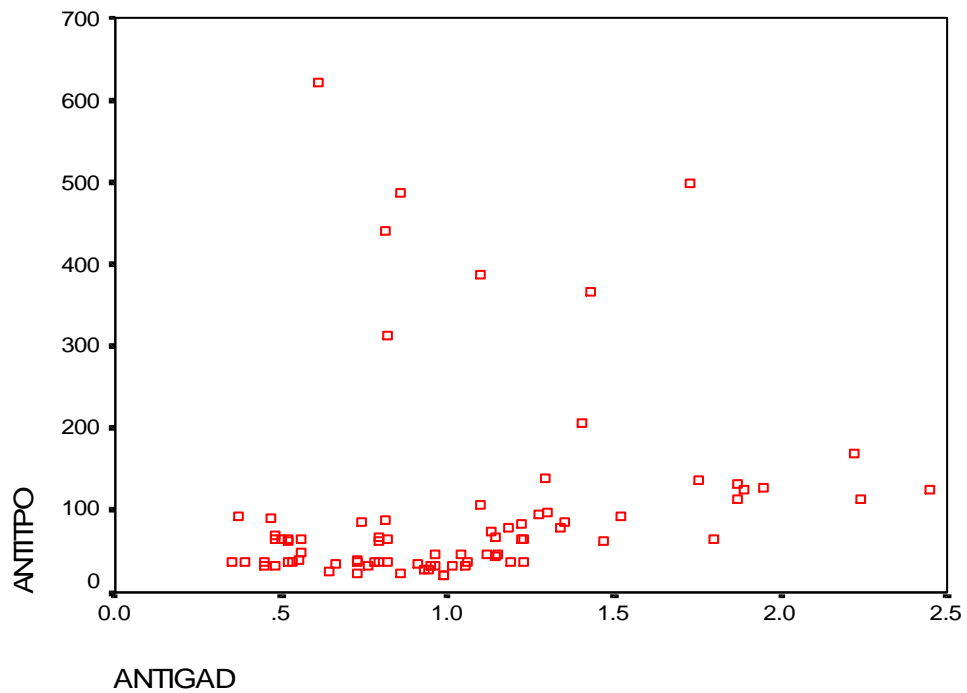


Figure 4.8 Scatter graph demonstrates the relationship between anti-TPO Abs and anti-GAD Abs levels

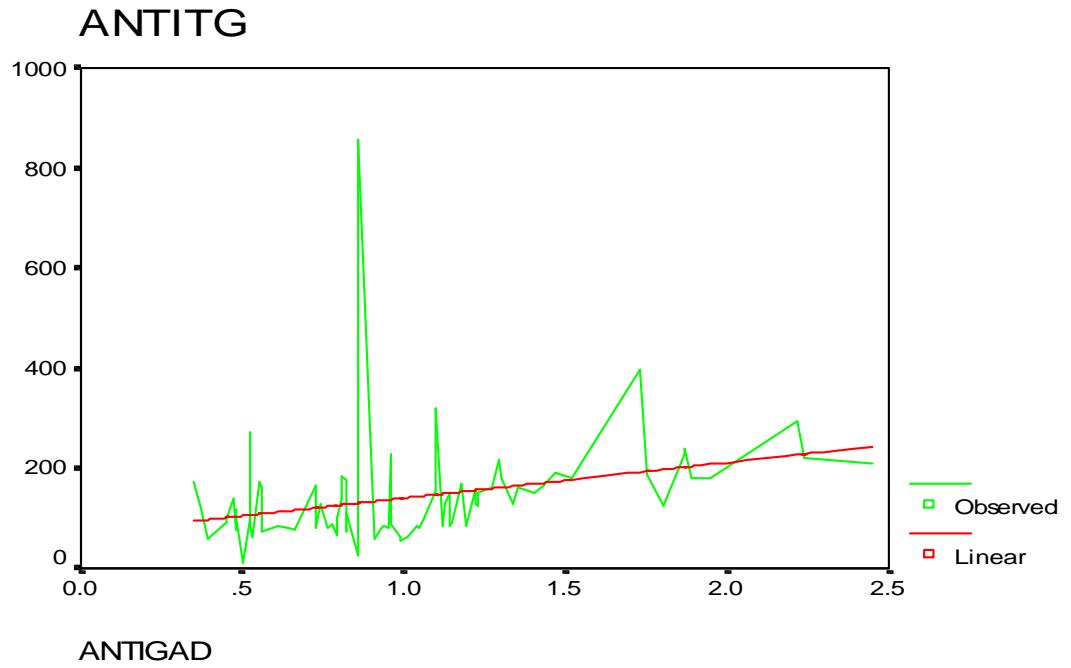


Figure 4.9 Regression line represents the prediction relation between anti-TG and anti-GAD Abs levels

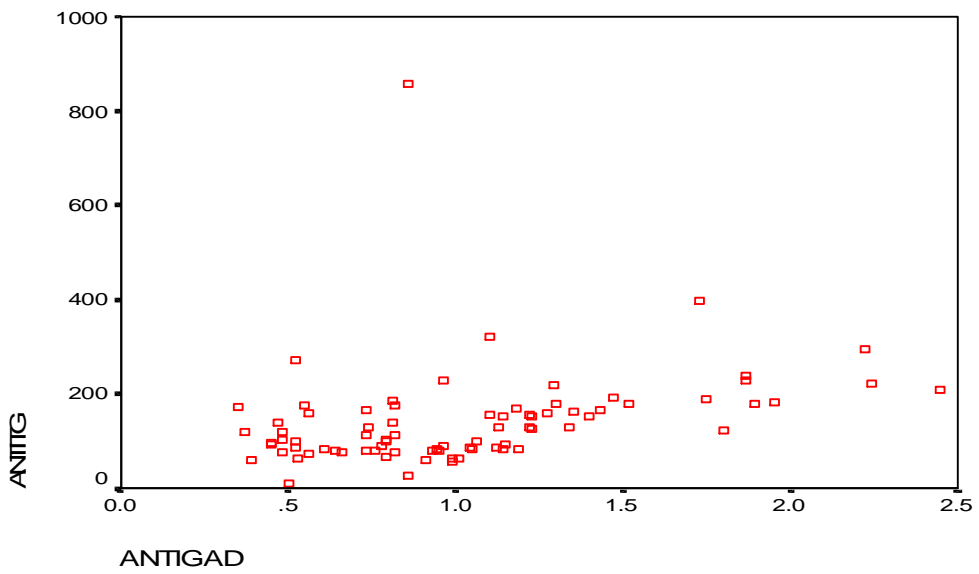


Figure 4.10 Scatter graph demonstrates the relationship between anti-TG Abs and anti-GAD Abs levels

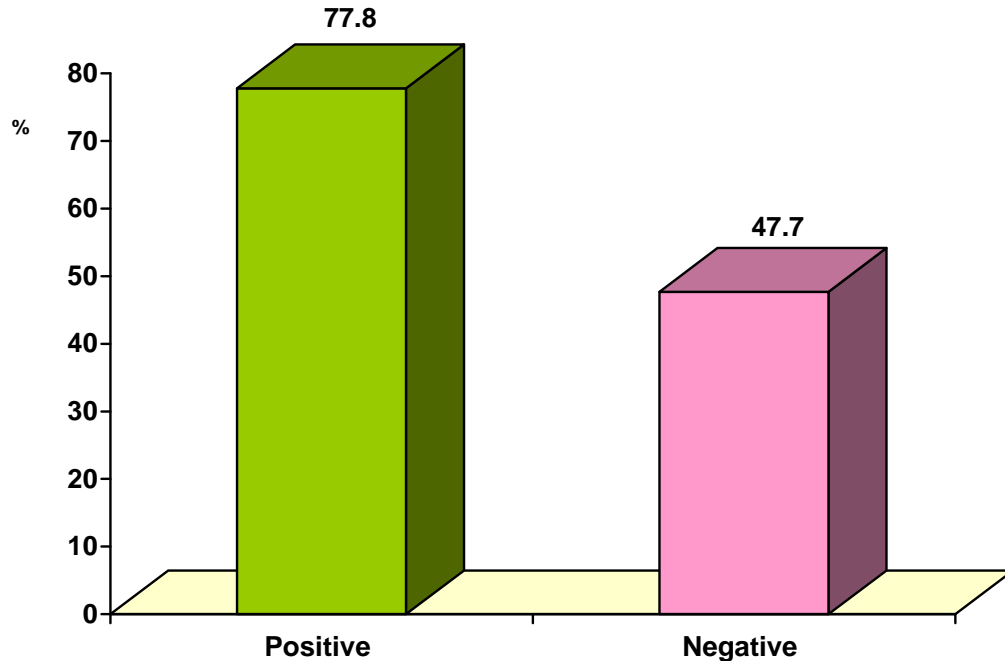


Figure 4.11 Distribution of study population by positive and negative cases of both anti-GAD and anti-thyroids Abs

4.3 Relationship of anti-TPO Abs

4.3.1 Relationship between anti-TPO Abs and personal factors

Table 4.4 revealed no statistically significant relationship between the gender of the subjects and anti-TPO Abs ($P= 0.14$), where are 40.9% of positive subjects are males and 59.1% of them are females. The negative males and females represent 55.6 and 44.4%, respectively (table 4.4 and fig. 4.12). Also there is no statistically significant differences between age groups according to the anti-TPO Abs ($P= 0.109$), but it is observed that the percentage of positive subjects are generally increased with increasing age (Figure 4.13).

Table 4.4 Relationship between anti-TPO Abs and personal variables (gender and age)

| Anti -TPO Abs Variable | Negative | | Positive | | Total | | p-value |
|---------------------------|----------|------|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| Gender | | | | | | | |
| Male | 20 | 55.6 | 18 | 40.9 | 38 | 47.5 | 0.14 |
| Female | 16 | 44.4 | 26 | 59.1 | 42 | 52.5 | |
| Total | 36 | 100 | 44 | 100 | 80 | 100 | |
| Actual Age Groups | | | | | | | |
| -10 | 12 | 33.3 | 6 | 13.6 | 18 | 22.5 | 0.109 |
| 10.01- 18 | 17 | 47.2 | 27 | 61.4 | 44 | 55 | |
| 18.01+ | 7 | 19.4 | 11 | 25 | 18 | 22.5 | |
| Total | 36 | 100 | 44 | 100 | 80 | 100 | |

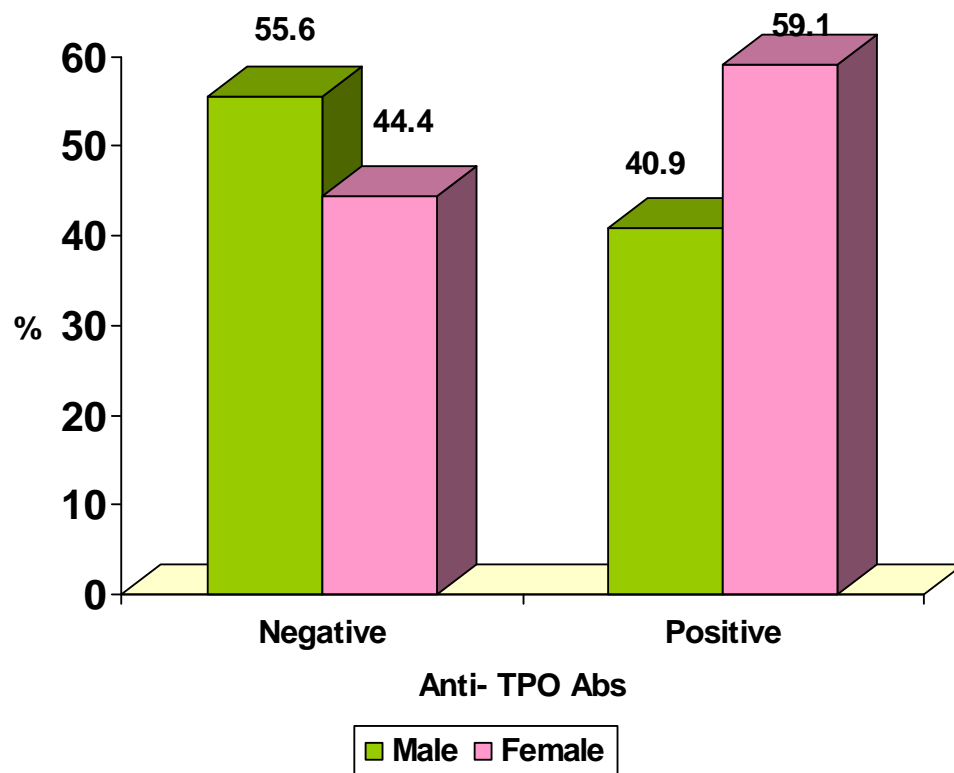


Figure 4.12 Distribution of the study population by the subjects gender and anti-TPO Abs

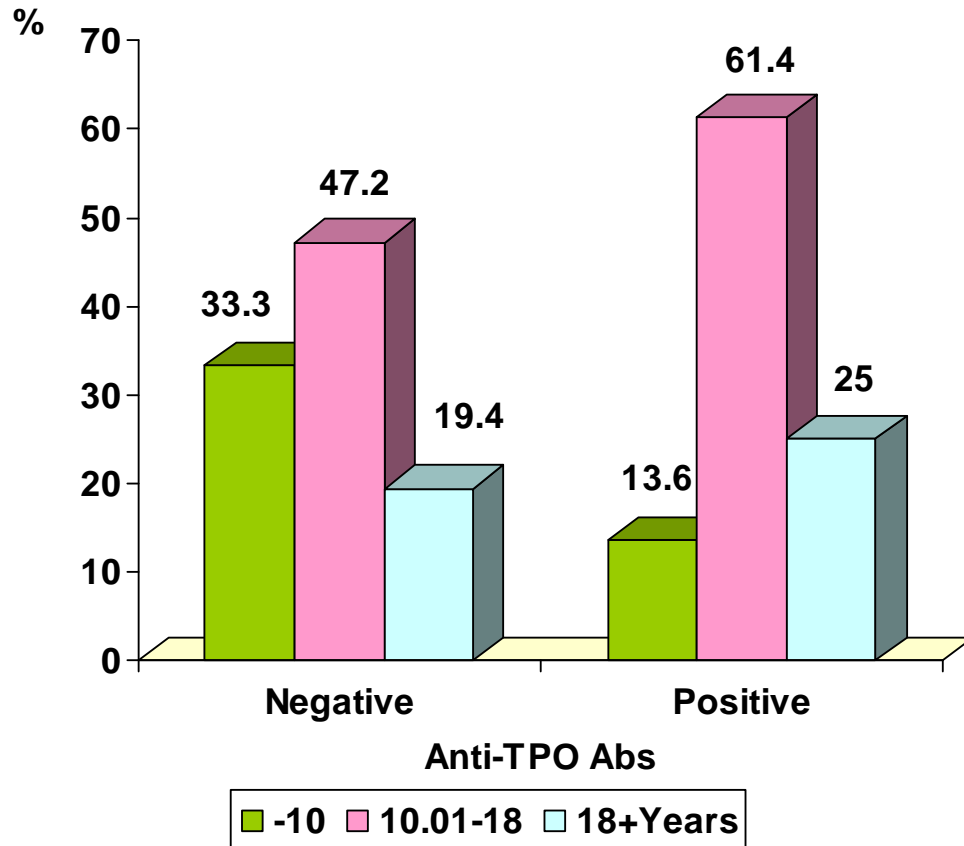


Figure 4.13 Distribution of the study population by age groups and anti-TPO Abs.

4.3.2 Relationship between Anti-TPO Abs and Duration of T1DM

Results data revealed a statistically significant correlation between the duration of T1DM and the serum level of anti-TPO Abs. So it is possible to predict the level of anti-TPO Abs (Y), by knowing the duration of DM type I (X) according to the regression equation, $[Y= 60.98+(0.26*X)]$, (P= 0.022). This is shown in Figures 4.14 and 4.15.

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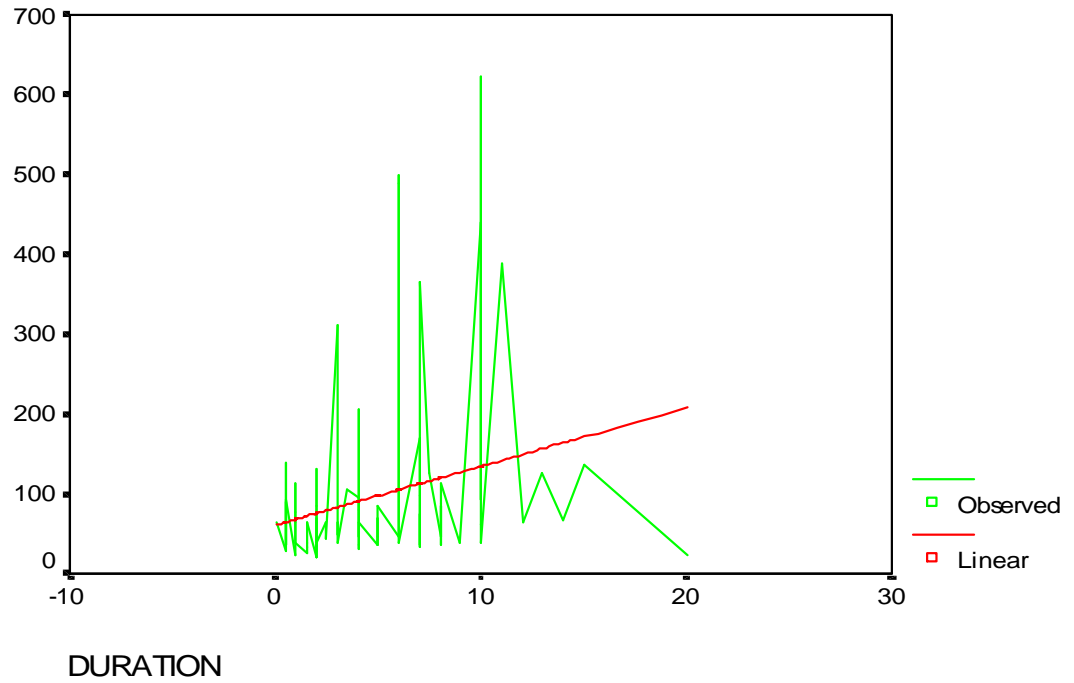


Figure 4.14 Regression line represents the prediction of relationship between duration of T1DM and anti-TPO Abs levels

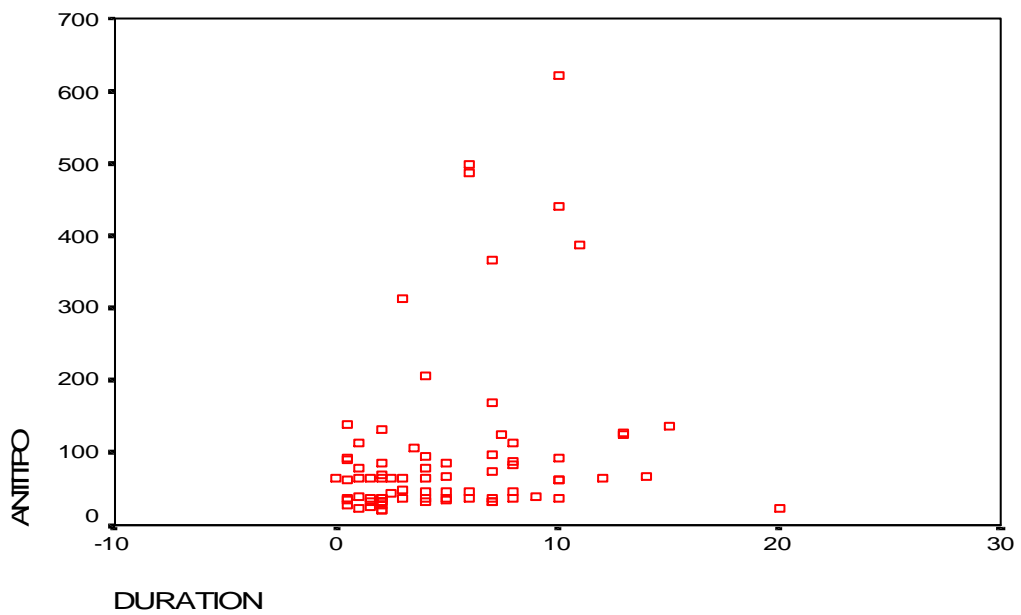


Figure 4.15 Scatter graph demonstrates the relationship between duration of T1DM and anti-TPO Abs levels

4.3.3 Relationship between anti-TPO Abs and hormones related to thyroid function

Results data showed no statistically significant relationship between anti-TPO Abs and both hormones related to thyroid function (FT4, and TSH). as demonstrated in (Table 4.5). The relationship between Anti-TPO and TSH is observed but it does not reach to statistical significant level ($X^2= 2.92$, $P= 0.087$).

Table 4.5 Relation between anti-TPO Abs and hormones related to thyroid function (FT4 and TSH)

| Anti -TPO Abs Variable | Negative | | Positive | | Total | | p-value |
|---------------------------|----------|------|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| FT4 | | | | | | | |
| Normal | 35 | 97.2 | 41 | 93.2 | 76 | 95 | 0.41 |
| High | 1 | 2.8 | 3 | 6.8 | 4 | 5 | |
| Total | 36 | 100 | 44 | 100 | 80 | 100 | |
| TSH | | | | | | | |
| Normal | 35 | 97.2 | 38 | 86.4 | 73 | 91.3 | 0.087 |
| High | 1 | 2.8 | 6 | 13.6 | 7 | 8.7 | |
| Total | 36 | 100 | 44 | 100 | 80 | 100 | |

4.3.4 Relationship between Anti-TPO Abs and Anti-TG Abs

Data results showed high statistically significant correlation between anti-TPO Abs and anti-TG Abs ($X^2=38.8$, $P= 0.000$). The percentage of subjects with positive anti-TPO Abs and anti-TG Abs was 88.6%, while the percentage of subjects of negative anti-TPO Abs and anti-TG Abs was 80.6% (Table 4.6). The equation of regression revealed high statistically significant correlation between anti-TPO Abs and anti-TG Abs, [$Y= 91.8+ (0.57*X)$], ($P= 0.000$) as shown in (Figures 4.16 and 4.17).

Table 4.6 Relationships between anti-TPO Abs and anti-TG Abs

| Anti -TPO Variable | Negative | | Positive | | Total | | p-value |
|-----------------------|----------|------|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| Anti-TG | | | | | | | |
| Negative | 29 | 80.6 | 5 | 11.4 | 34 | 42.5 | 0.000** |
| Positive | 7 | 19.4 | 39 | 88.6 | 46 | 57.5 | |
| Total | 36 | 100 | 44 | 100 | 80 | 100 | |

** = High Significant

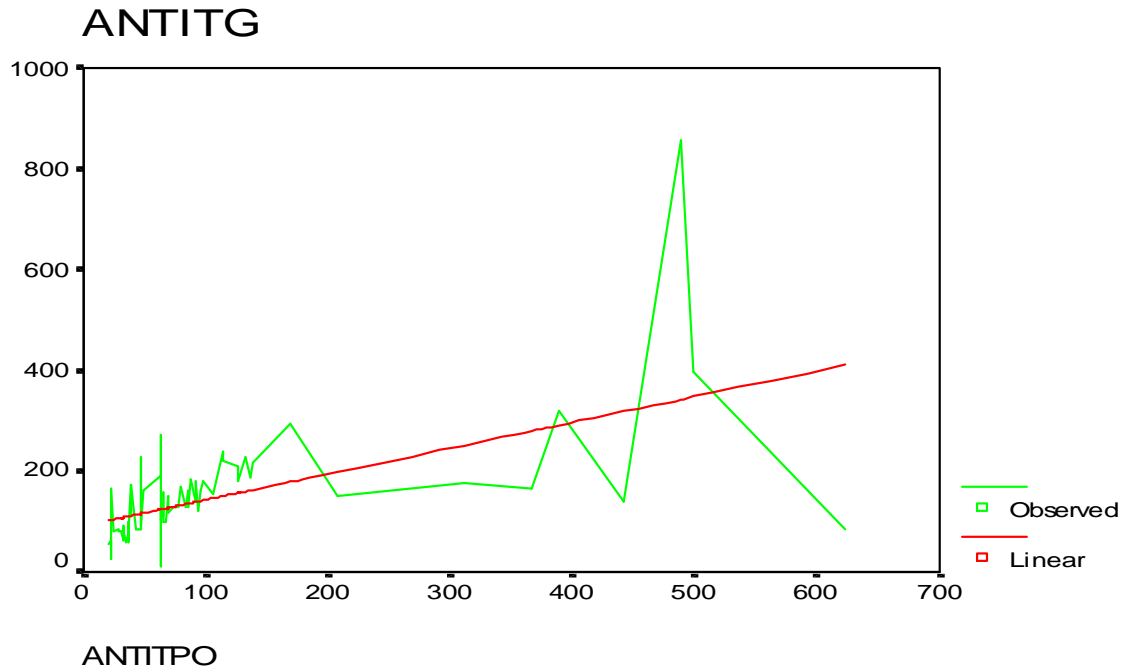


Figure 4.16 Regression line represents the prediction of relationship between anti-TPO Abs and anti-TG Abs levels

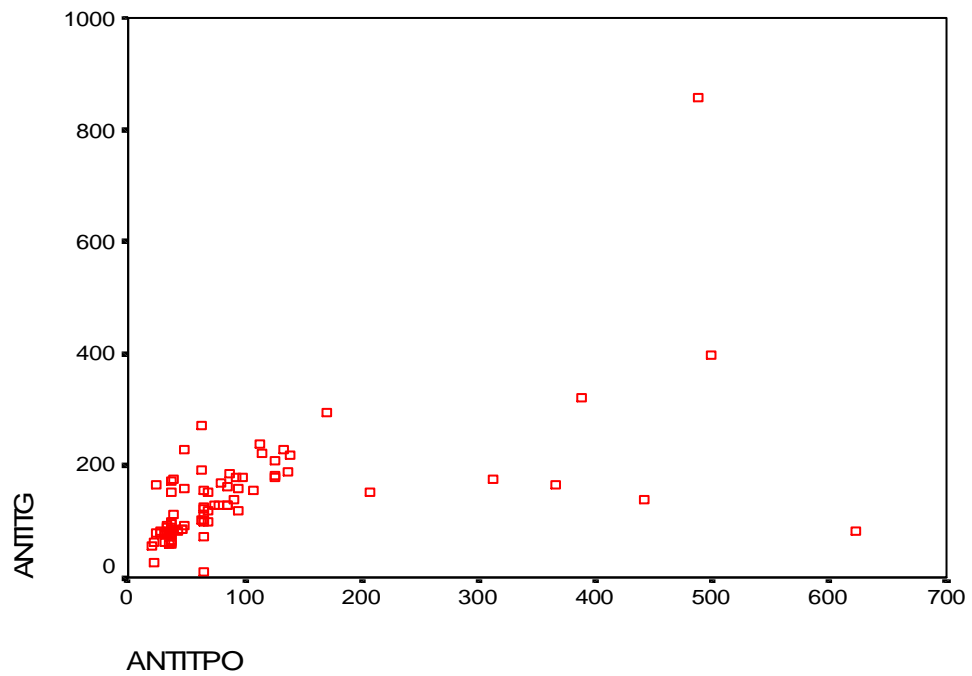


Figure 4.17 Scatter graph demonstrates the relationship between anti-TPO Abs and anti-TG Abs levels

4.4 Relationship of anti-TG Abs

4.4.1 Relationship between anti-TG Abs and personal factors

Data results in table 4.7 revealed a weak relationship between the gender of subjects and anti-TG Abs ($X^2= 3.04$, $P= 0.064$), where are 39.1% of positive subjects are males and 60.9% of them are females. The negative males and females represent 58.8 and 41.2%, respectively (table4.7 and fig.4.18). Also there was no statistically significant differences between age groups according to the anti-TG Abs ($P= 0.38$). The percentage of positive subjects was generally increased with increasing age (table 4.7 and Fig. 4.19).

Table 4.7 Relation between anti-TG Abs and personal variables (gender and age)

| Anti –TG Variable | Negative | | Positive | | Total | | p-value |
|-------------------|----------|------|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| Gender | | | | | | | |
| Male | 20 | 58.8 | 18 | 39.1 | 38 | 47.5 | 0.064 |
| Female | 14 | 41.2 | 28 | 60.9 | 42 | 52.5 | |
| Total | 34 | 100 | 46 | 100 | 80 | 100 | |
| Actual Age | | | | | | | |
| -10 | 10 | 29.4 | 8 | 17.4 | 18 | 22.5 | 0.38 |
| 10.01- 18 | 18 | 52.9 | 26 | 56.5 | 44 | 55 | |
| 18.01+ | 6 | 17.6 | 12 | 26.1 | 18 | 22.5 | |
| Total | 34 | 100 | 46 | 100 | 80 | 100 | |

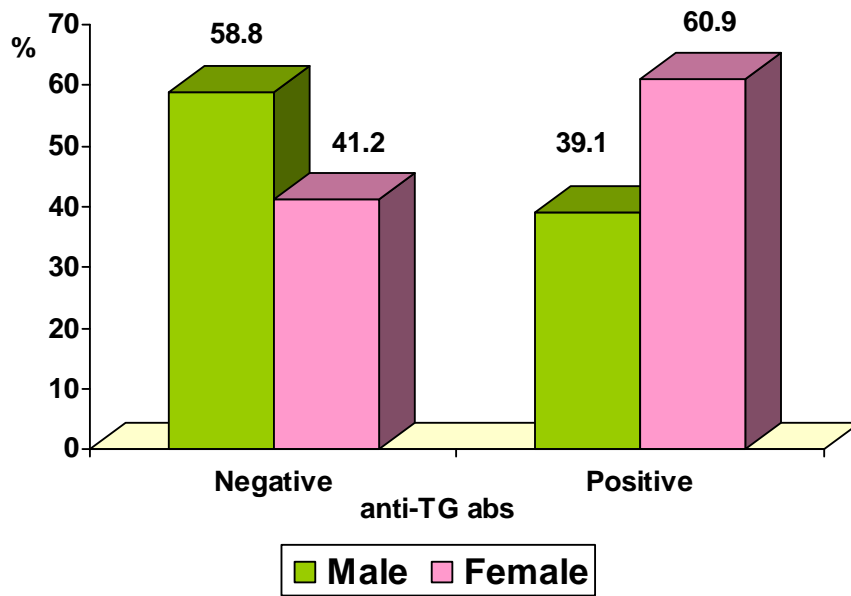


Figure 4.18 Distribution of the study population by anti-TG Abs and gender

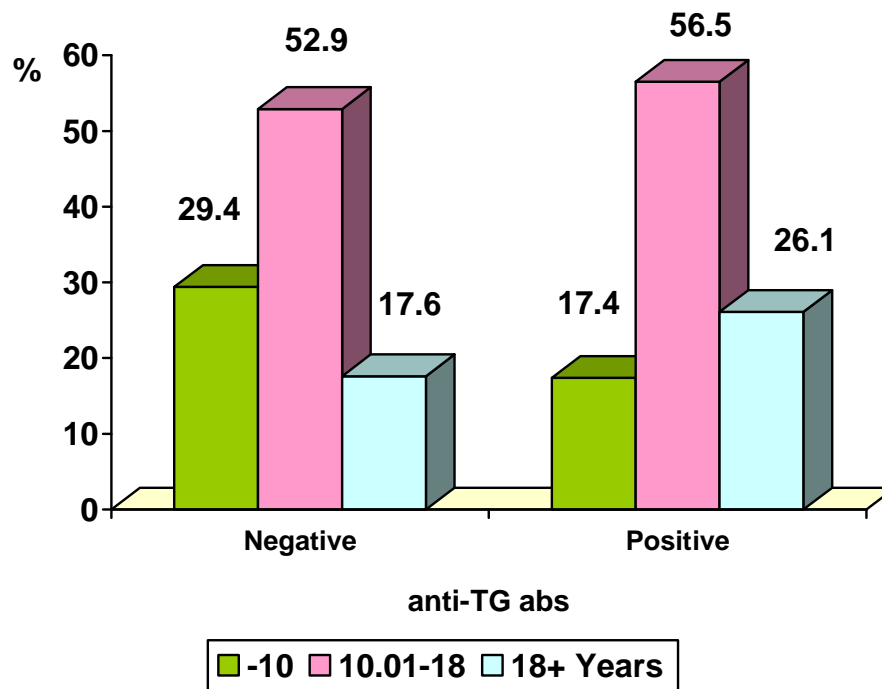


Figure 4.19 Distribution of the study population by anti-TG Abs and age groups

4.4.2 Relationship between anti-TG Abs and duration of T1DM

Results revealed no statistically significant correlation between the duration of T1DM and the serum level of anti-TG Abs. So it is not possible to predict the level of anti-TG Abs (Y), by knowing the duration of T1DM (X) according to the regression equation (P= 0.99)

4.4.3 Relationship between anti-TG Abs and thyroid related hormones

Table 4.8 showed no statistically significant relation between anti-TG Abs and both hormones related to thyroid function (FT4 and TSH) (P= 0.47, P= 0.11, respectively).

Table 4.8 Relation between anti-TG Abs and thyroid related hormones (FT4, and TSH)

| Anti -TG Abs Variable | Negative | | Positive | | Total | | p-value |
|--------------------------|----------|------|----------|------|-------|------|---------|
| | No. | % | No. | % | No. | % | |
| FT4 | | | | | | | |
| Normal | 33 | 97.1 | 43 | 93.5 | 76 | 95 | 0.47 |
| High | 1 | 2.9 | 3 | 6.5 | 4 | 5 | |
| Total | 34 | 100 | 46 | 100 | 80 | 100 | |
| TSH | | | | | | | |
| Normal | 33 | 97.1 | 40 | 87 | 73 | 91.3 | 0.11 |
| High | 1 | 2.9 | 6 | 13 | 7 | 8.7 | |
| Total | 34 | 100 | 46 | 100 | 80 | 100 | |

Chapter 5

Discussion

This study investigated the existence of anti-GAD antibodies and thyroid autoantibodies in T1DM patients. Also the effect of gender variation, duration of disease and others risk factors for both thyroid disease and T1DM among Gaza children, adolescent and adult up to 25 years old have been also investigated. To achieve these objectives anti-GAD antibodies , anti- TPO antibodies, anti-TG antibodies, FT4,and TSH were measured for 80 patients of T1DM (38 males and 42 females) from central diabetic clinic in Gaza city.

As T1DM is often associated with other endocrine autoimmune disorders, so circulating auto-antibodies could be a hallmark of clinical or sub-clinical autoimmune polyendocrine disease, particularly in T1DM,autoimmune thyroid disease.

Many studies postulated that the detection of thyroid antibodies might reflect the developing of other autoimmune disease such as T1DM. This led to suggestion that the search for thyroid antibodies could be a good prognosis for other autoimmune disease especially T1DM.

The present study in general is important in order to identify the extent of the disease in the community and to verify the etiological causes which leading to mortality and morbidity among the population, also to evaluate the determinants of preventing and control the disease and to find the relative risk factors which mainly associated with the disease.

5.1 Thyroid dysfunction incidence

Investigating the level of thyroid hormones (TSH and FT4) for all subjects has shown that 91.3% had a normal TSH level while 8.7% had high level. Patients with high levels of TSH could be either due to hypothyroidism or hyperthyroidism but the level of TSH is high due to medication, that means

if the medication treatment was high dose TSH level became higher than normal to compensated treatment effect. Also 95% of the subjects had normal FT4 level while 5% had high level, which mean they have hyperthyroidism.

The results of TSH and FT4 indicate that 5-10 % of our type 1DM patients had thyroid disorder. This agreed with other studies in type 1 DM patients in other populations (**Radaideh et al., 2003 and Sarah et al., 2005**).

The results showed that 36.2 % of our T1DM patients were positive anti-TPO Abs, 50% were positive anti-TG Abs, and 45% were positive anti-GAD Abs. The investigation of the levels and pancreas of thyroid autoantibodies were found to be significant in T1DM patients. These results are consistent with recent study by **Sarah et al., (2005)** who found the incidence rate of thyroid disease in their study was 7.8% for anti-TPO Abs and 66% for anti-GAD Abs.

In similar study in ethnically related population **Radaideh et al., (2003)** found that 8.9% of T1DM cases had thyroid dysfunction which is similar to our results. However 9.2% patients were found to have thyroid autoantibodies positive which is different from our study. This could be due to difference in disease duration in the two population groups. Other population studies like (**Kalicka et al 2003 and Rodacki et al., 2004**) found that T1DM patients had 45.8% positive anti-GAD Abs. and 34.7%had positive anti-TPO Abs.

As was mentioned before T1DM is an autoimmune disease which result from β cell destruction due to the production of a number of antibodies against it e.g. anti-GAD Abs. This explains the high percentage of anti-GAD Abs in our patient group. As thyroid autoimmune disease is the most frequent concomitant disease to T1DM, the results are consistent with the expectation that thyroid autoantibodies are quite frequent in T1DM patients and this agrees with other population studies (**Barova et al., 2004**).

5.2 Anti-GAD antibodies in T1DM

The autoimmune mechanism is activated by specific antigens which are tested by specific antibodies. Destruction of β cells of pancreatic islets in

T1DM, which is an autoimmune mechanism, is mediated by specific antigens and the most existent antigen is GAD, but GAD is present not only in β cells but also in brain (**Henry et al., 1992**), so we can find its antibody in stiff man syndrome (rare neurological disorder)

5.2.1 Anti-GAD Abs and personal factors

Correlation between anti-GAD Abs and personal factors revealed a significant relationship between gender of subjects and anti-GAD Abs, where 61.1% of positive subjects were males, and 38.9% of them were females. In spite of the absence of statistically significant differences between age groups according to the Anti-GAD Abs, it has been observed that the percentage of positive subjects is increased with increasing age (Table 4.1). This could be due to duration of diabetes and not just due to age difference. In accordance with our study **Rodacki et al., (2004)** in Brazil found no correlation between anti-GAD Abs and age at diagnosis and the prevalence of anti-GAD antibodies was 53.8% in males and 32.3% females. In contrast **Prazny et al., (2005)** has found no difference in anti-GAD antibodies between males and females. The difference between these studies could be due to environmental, genetic or ethnic factors.

5.2.2 Anti GAD Abs and duration of T1DM

Correlation analysis between anti-GAD antibodies and duration of diabetes type 1 has revealed significant correlation between the mean concentration of anti-GAD Abs with the disease duration. In accordance with our results **Sarah et al., (2005)** showed the same results, but in contradiction **Rodacki et al., (2004)** found that duration of the disease did not affect significantly the prevalence of anti-GAD Abs positivity or its titers after one year of diagnosis. In addition **Elkadhi et al., (2002)** in Tunis found that the duration of the disease has an influence on the levels of anti-GAD Abs. The existing different relation could be due to longer disease duration. Therefore

anti-GAD antibodies need to be measured at the onset of diagnosis and followed by regular measures in order to establish any defined correlations.

5.2.3 Anti-GAD Abs and hormones related to thyroid function

The relationship between anti-GAD Abs and hormones related to thyroid function (FT4, and TSH) also has been studied. Our results has shown no statistically significant relation between anti-GAD Abs and both Hormones related to thyroid function (FT4, and TSH) as demonstrated in (Table 2). **Radaideh et al., (2003)** found 7(8.9%) of type 1 DM patients (who have highly prevalence of anti-GAD Abs) had thyroid dysfunction, 4 of these were diagnosed as subclinical hypothyroidism, whereas the other 3 had hypothyroidism. Their results were similar and confirmed our results. This could be explained on the basis thyroid hormone levels are good indicators for actual thyroid disease but do not predict thyroid disease like thyroid auto antibodies.

5.2.4 Anti-GAD Abs and thyroid antibodies

There was a highly significant correlation between anti-GAD Abs and each anti-TPO Abs, or/and anti-TG Abs. The percentage of subjects with positive anti-GAD Abs and anti-TPO Abs was 75%, while the percentage of subjects with negative anti-GAD Abs and anti-TPO Abs was 62.4%. In addition the percentage of subjects with positive anti-GAD Abs and anti-TG Abs was 77.8%, while the percentage with negative anti-Gad and anti-TG was 59.1% as shown in (Table 3). In accordance with our study **Barova et al., (2004)** found that there was high frequency of thyroid antibodies in anti-GAD Abs positive T1DM patients.

The patients with T1DM were further grouped according to their anti-GAD Abs status. The anti-GAD Abs positive patients had a higher prevalence of anti-TG Abs than the anti-GAD Abs negative patients as well as anti-TPO Abs, **Prazny et al., (2005)** also found the same result, this is consistent with

the findings that the most frequently concomitant disease of T1DM is autoimmune thyroid disease (**Kinova et al 1998**).

5.3 Anti-TPO Abs

TPO Abs appear to be involved in the tissue destructive processes associated with the hypothyroidism, such as that observed in Hashimoto's and atrophic thyroiditis, and it is against thyroid peroxidase enzyme and microsomal antigen.

5.3.1 Anti-TPO Abs and personal factor

Correlation analysis between anti-TPO Abs and personal factors has shown no statistically significant relationship between the gender of subjects and anti-TPO Abs, where 40.9% of positive subjects were males, while 59.4% of them were females. Negative males and females were 55.6 and 44.4%, respectively. Also there was no statistically significant differences between age groups according to the anti-TPO Abs, but it was observed that the percentage of positive subjects were increased with increasing age, while **Kordonouri et al.,(2005)** has found that 15.4% has increased anti-TPO antibodies and females had more frequently increased anti-TPO antibodies than males. In an other study **Holl et al., (1999)** found that the prevalence of elevated thyroid antibodies increased dramatically with age, patients older than 10 years. Females were significantly more affected than males. As we observed there was a correlation between increasing in age and the increasing of anti-TPO antibodies titer and we assume this is due to duration of disease as explained later.

5.3.2 Anti-TPO Abs and duration of T1DM

Our results revealed a correlation between anti-TPO antibodies and duration of T1DM; it means that the higher anti-TPO antibodies reflect the

severity of disease which means long duration of the disease. Data showed statistically significant correlation between the duration of diabetes mellitus type 1 and the serum level of anti-TPO Abs. So it is possible to predict the level of anti-TPO Abs by knowing the duration of T1DM according to the regression equation.

In accordance with our study **Kordonouri et al., (2005)** found in their study that anti-TPO and anti-TG antibodies were significantly increased in value and incidence in their subjects who were T1DM during 5 years.

5.3.3 Anti-TPO Abs and hormones related to thyroid function

Data showed no statistically significant relation between anti-TPO Abs and both hormones related to thyroid function (FT4, and TSH) as demonstrated in (Table 4.5). **Kalicka-Kasperczyk et al., (2003)** found positive anti-TPO Abs titer in 34.7% patients of his population with T1DM. Thyroid dysfunction was detected in 5.05% patients of positive anti-TPO Abs, a similar result to our study, which showed that 13.6% of positive anti-TPO Abs patients had significantly higher level of TSH, while 2.8% of negative anti-TPO Abs patients had significantly higher level of TSH. Also, 6.8% of positive anti-TPO Abs patients had significantly higher level of FT4, while 2.8% of negative anti-TPO Abs patients had significantly higher level of FT4. Therefore we assume that anti-TPO Abs or anti-TG antibodies or both can be detected long before the changes of TSH and thyroid hormone levels occur. Thus, the determination of these antibodies might be useful for early diagnosis of the disease before thyroid dysfunction develops. **Barova et al (2004)** found that the prevalence of thyroid dysfunction in their study population was 9%, which is equivalent and support our findings.

5.3.1 Anti-TPO Abs and anti-TG Abs

We found a strong correlation between anti-TPO and anti-TG antibodies, where the percentage of subjects with both antibodies positive was 88.6% and the percentage of negative anti-TG Abs in subjects who were

negative anti-TPO Abs were 80.6%. **Rattarasarn et al., (2000)** found that anti-TG Abs and anti-TPO Abs were positive in 18% and 30% patients, respectively. **Menon et al (2001)** found also anti-TPO in 54.3% and anti-TG Abs in 31.4%.

The results of previous studies made support for our study which revealed a strong correlation between these two antibodies. Because production of thyroid hormones depends on many factors which essentially for production of thyroid hormones and the most important one are thyroid peroxidase enzyme and thyroglobulin protein which in autoimmune disorder the body produce autoantibodies against it anti-TPO and anti-TG, and because defect often happen in both, so there was association between these antibodies but the higher rate of finding anti-TPO Abs was due to enzyme defect was slightly more. On the other mean Abs to both autoantigens were frequently present in the same patient. One explanation for their concurrence was that they arise independently in response to the release of their respective autoantigens after thyroid cell damage. An alternative explanation postulates a role for cross-reactivity in the autoimmune response to TG and TPO (**Francesco et al., 2003**).

5.4 Thyroglobulin

Thyroglobulin (TG) is a large glycoprotein (molecular weight: 660000) with 2 polypeptide chains of approximately 2768 amino acids each. It functions both as a pro-hormone and storage hormone for thyroid hormones(**McLachlan., et al 2004**). TG is one of the thyroid auto antigens recognized in patients with autoimmune thyroid disease (AITD). Antibodies to TG (TG Abs) were present in the serum of patients with AITD and are also sometimes present in healthy euthyroid subjects, several TG fragments capable of inducing a T-cell response (**David et al., 2001**).

5.4.1 Anti-TG Abs and personal factor and duration of T1DM

The results revealed that there was weak relationship between the gender of subjects and anti-TG Abs, where 39.1% of positive subjects were males, and 60.9% of them were females. Also there were no statistically significant differences between age groups according to the anti-TG Abs, but it is observed that the percentage of positive subjects is increased with increasing age. In addition there was no statistically significant correlation between the duration of T1DM and the serum level of anti-TG Abs. found in their. **Jennifer M et al., (2005)** found in their study group that 29% of subjects were positive for either anti- TPO Abs and/or anti-TG Abs, and the thyroid autoantibody–positive subjects were more likely to be female, older, and with a longer duration of diabetes. Our results had the same trend in general but the difference was in existence proportion. This may be due to ethnic, and environmental variables. The explanation of increased antibodies with age could be due to severity of the disease and because in our study age group of subjects less than 25 years and many of them had recently onset of disease. So the results did not represent the real relation with duration of disease, and hence further studies are needed.

5.4.2 Anti-TG Abs and hormones related to thyroid function

Likewise to the correlation between anti-TPO Abs and hormones related to thyroid function (**Hansen et al., 1999**) found that A high proportion of T1DM patients without any clinical signs of thyroid disease have positive anti-TG Abs. That results was same as and support our results. The results showed that there was no statistically significant relation between Anti-TG and both hormones related to thyroid function (FT4, and TSH).

Chapter 6

Conclusions and Recommendations

6.1 Conclusions

1. T1DM is strongly associated with anti-GAD Abs as the existence of the antibody was >45% of study subjects
2. In the Gaza city t T1DM as an autoimmune disease can be associated with other autoimmune endocrine disorder, as a strong relation between anti-GAD Abs and anti-thyroid Abs was detected
3. This study confirms the association between autoimmune thyroid dysfunction and T1DM.
4. The diagnosis of AITD should be based on the assessment of autoantibodies to anti-TPO, and anti-TG, These antibodies are positive in some patients of T1DM whereas hormones related to thyroid function (FT4 , TSH) found normal level but some patients had high or low level of these Hormones.
5. The association of autoimmune disease may influence the subjects of diabetes by impairing function of the respective organ, so our result showed that thyroid diseases is found and associated with T1DM.
6. Biochemical thyroid dysfunction and thyroid autoimmunity were evident in T1DM who were apparently euthyroid.
7. Thyroid autoimmunity seems to be particularly more common in girls with T1DM.
8. The presence of thyroid antibodies is associated with a higher frequency predict a higher risk for thyroid dysfunction in T1DM patients.

6.2 Recommendations

1. Measurement of anti-GAD Abs could have a high possibility to predict if the diabetic patient with T1DM.

2. Early detection of AITD and bodies for early age children who with relation degree to T1DM patient may be detects the prognosis of these children to have DM or thyroid dysfunction in future.
3. Screening for thyroid disease should be performing at diabetes onset in all patients with T1DM.
4. If the initial thyroid screening is positive, annual laboratory anti-TG Abs, TPO Abs, TSH, and FT4 examination are necessary in order to detect early thyroid dysfunction and initiate treatment..
5. There is lack of studies in this area; so much more research is needed to identify some health problems in our country and to performe solutions for it, and that will developing health society in the Gaza Strip.

Chapter 7

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Date: 30/10/2005

التاريخ: 2005/10/30

Mr./ Hossam Qwader

السيد: حسام قويدر

I would like to inform you that the committee
has discussed your application about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم
حول:-

**Thyroid and Glutamic Acid Decarboxylase
Autoantibodies Status of Type Diabetes
Mellitus Subjects in Gaza.**

In its meeting on October 2005
and decided the Following:-

و ذلك في جلستها المنعقدة لشهر أكتوبر 2005

و قد قررت ما يلي:-

To approve the above mention research study.

الموافقة على البحث المذكور عالياه.

Signature

توقيع

Member

Member



عضو

عضو

Chairperson

Conditions:-

- ❖ Valid for 2 years from the date of approval to start.
- ❖ It is necessary to notify the committee in any change in the admitted study protocol.
- ❖ The committee appreciate receiving one copy of your final research when it is completed.

بسم الله الرحمن الرحيم

حفظه الله

السيد مدير عام الرعاية الأولية

تحية وتقدير

الموضوع / الموافقة على إجراء بحث طالب ماجستير

أتقدم لسيادتكم بطلبي هذا حيث أنني بصدد إكمال متطلبات البحث لنيل درجة الماجستير في

التحاليل الطبية بعنوان

Thyroid and GAD autoantibody status of type 1 D.M. subjects in Gaza

وهذا يتطلب زيارتي إلي عيادة السكر والغدد بمركز شهداء الرمال لتحديد العينة البحثية

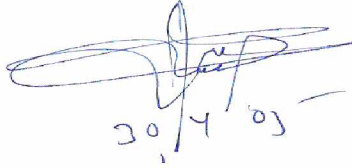
من خلال ملفات مرضى السكر من النوع الأول لذا أرجو السماح بتقديم التسهيلات اللازمة

من خلال القائمين على هذا الموضوع .

ولكم جزيل الشكر

مقدمة الطالب

حسام يوسف قويدر


30/4/05

Handwritten notes and signatures:

وزير الصحة
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بسم الله الرحمن الرحيم

السيدة/ مدير دائرة المختبرات وبنوك الدم حفظها الله

تحية وتقدير

الموضوع / الموافقة على إجراء بحث طالب ماجستير

أتقدم لسيادتكم بطلبي هذا حيث أنني بصدد إكمال متطلبات البحث لنيل درجة الماجستير في

التحاليل الطبية بعنوان

Thyroid and GAD autoantibody status of type I D.M. subjects in Gaza

أرجو السماح لي بإجراء بعض الفحوصات اللازمة لاتمام هذا البحث وحيث أن عينة البحث

تقدر بحوالي 80 عينة ويلزمها الفحوصات التالية :-

- 1- Anti. GAD65 .
- 2- Thyroid antibodies .
- 3- TSH .


لذا أرجو السماح لي بالعمل بالمختبر المركزي حسب الإمكانيات المتوفرة .
مع العلم أنني سأتكفل بتوفير المواد الناقصة .

ودمتم ذخراً للمسيرة التعليمية .

ولكم جزيل الشكر

مقدمة الطلب

حسام يوسف قويدر


30/4/05

المدير
لما في هـ لظروف استوزة
د. س. س.
30/4/05

صادر: 30/4/05
وارد: 30/4/05
تاريخ: 30/4/05