INTRODUCTION

Vitamin D is a fat-soluble vitamin which is predominantly synthesized in the skin, it is considered one of the natural immune modulators and a regulator of various immune-mediated processes ***(Agmon-Levin et al., 2013)***.

The mechanisms underlying the assumption that vitamin D is linked with autoimmunity are not clear but probably are associated with its anti-inflammatory and immune modulatory functions ***(Lacka and Maciejewski, 2013).***

Vitamin D deficiency is a global health problem**.** Over a billion people worldwide are vitamin D deficient or insufficient. Understanding of the role of vitamin D has been evolving since its discovery in the early 20th century from being a simple vitamin to a steroid pro-hormone. It has been recognized to be involved in various immune functions as well as bone and muscle development. Vitamin D deficiency has been shown to be associated with autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), multiple sclerosis (MS) and type 1 diabetes (T1DM), and that vitamin D supplementation prevents the onset and/or development of these autoimmune diseases ***(Naeem, 2010).***

Hashimoto's thyroiditis (HT), also called chronic lymphocytic or autoimmune thyroiditis, is part of the spectrum

of chronic autoimmune thyroid diseases (AITD) ***(Caturegli, 2014).***

It is characterized by female preponderance, enlargement of the thyroid gland, thyroid autoantibody production and lymphocytic infiltration, associated with various degrees of thyroid hypofunction. Although the exact mechanism of progressive thyroid tissue destruction is not clear, HT is regarded as a disorder of T cell-mediated immunity caused by an interaction between susceptibility genes, e.g., cytotoxic T-lymphocyte associated-4 (CTLA-4), human leukocyte antigen (HLA), TSH receptor (TSHR) and environmental factors, both of which are not yet completely understood ***(Lacka and Maciejewski, 2013).***

Many environmental factors associated with the development of autoimmune thyroid disease including stress, smoking, excess iodine, selenium deficiency, vitamin D deficiency, irradiation, pollutants and infections ***(Weetman, 2011).***

It was reported that patients with Hashimoto's thyroiditis, an autoimmune thyroid disease had lower vitamin D levels ***(Tamer et al., 2011).*** Several studies have revealed low serum 25[OH]D levels in patients with HT indicating an association between vitamin D deficiency and thyroid autoimmunity ***(Bozkurt et al., 2013).***

AIM OF THE WORK

The aim of this work is to assess active vitamin D status in some Egyptian patients with autoimmune hypothyroidism proved by assay of TPO in those patients.

Chapter 1

HYPOTHYROIDISM

Hypothyroidism is a disorder that occurs when the thyroid gland does not make enough thyroid hormone to meet the body's needs. Thyroid hormone regulates metabolism, the way the body uses energy, and affects nearly every organ in the body. Without enough thyroid hormone, many of the body's functions slow down.The thyroid gland makes two thyroid hormones, tri-iodothyronine (T3) and thyroxine (T4). T3 is made from T4 and is the more active hormone, directly affecting the tissues ***(Adorini and Penna, 2008).***

Thyroid hormones affect metabolism, brain development, breathing, heart and nervous system functions, body temperature, muscle strength, skin dryness, menstrual cycles, weight, and cholesterol levels. Thyroid hormone production is regulated by thyroid-stimulating hormone (TSH), which is made by the pituitary gland in the brain. When thyroid hormone levels in the blood are low, the pituitary releases more TSH. When thyroid hormone levels are high, the pituitary responds by dropping TSH production ***(Golden et al., 2009).***

Hypothyroidism may occur as a result of primary gland failure or insufficient thyroid gland stimulation by the hypothalamus or pituitary gland. Primary gland failure can result from congenital abnormalities, autoimmune destruction

(Hashimoto disease), iodine deficiency, and infiltrative diseases ***(Boucai et al., 2011)***.

Iatrogenic forms of hypothyroidism occur after thyroid surgery, radioiodine therapy, and neck irradiation ***(Devdhar et al., 2007).***

Disorders generally associated with transient hypothyroidism include postpartum thyroiditis, subacute thyroiditis, silent thyroiditis, and thyroiditis associated with thyroid-stimulating hormone (TSH) receptor-blocking antibodies ***(Devdhar et al., 2007).***

Central causes of hypothyroidism typically present with other manifestations of hypothalamic or pituitary dysfunction, and are characterized by inappropriately normal or low levels of TSH relative to insufficient thyroid hormone. Drugs classically associated with thyroid dysfunction include lithium, amiodarone, interferon alfa, interleukin-2, and tyrosine kinase inhibitors ***(Pérez López et al., 2011).***

Hypothyroidism can also be secondary—that is, the thyroid gland itself is normal, but it receives insufficient stimulation because of low secretion of thyrotropin (ie, thyroid-stimulating hormone [TSH]) from the pituitary gland. In tertiary hypothyroidism, inadequate secretion of thyrotropin-releasing hormone (TRH) from the hypothalamus leads to

insufficient release of TSH, which in turn causes inadequate thyroid stimulation ***(Devdhar et al., 2007).***

Clinical Presentation:-

Thyroid hormone receptors regulate many key physiologic processes. Consequently, hypothyroidism may result in a myriad of clinical signs and symptoms. The severity of these manifestations generally reflects the degree of thyroid dysfunction and the time course of development of hypothyroidism. Symptoms commonly associated with hypothyroidism are often nonspecific. These include weight gain, fatigue, poor concentration, depression, diffuse muscle pain, and menstrual irregularities. Symptoms with high specificity for hypothyroidism include constipation, cold intolerance, dry skin, proximal muscle weakness, and hair thinning or loss ***(Wiersinga, 2012).***

Symptoms of hypothyroidism may vary with age and sex. Infants and children may present more often with lethargy and failure to thrive. Women who have hypothyroidism may present with menstrual irregularities and infertility. In older patients, cognitive decline may be the sole manifestation. Examination findings associated with hypothyroidism include but are not limited to goiter, delayed relaxation phase of deep tendon reflexes, thin or brittle hair, dry skin, and peripheral edema. Common electrocardiography findings include bradycardia, flattened T waves, and low voltage. Patients with

severe hypothyroidism may present with pericardial effusion, pleural effusion, megacolon, hemodynamic instability, and coma. The clinical presentation is often confused with septic shock. Myxedema coma, which represents severe physiologic decompensation resulting from hypothyroidism, occurs rarely, with an annual incidence of 0.22 per million ***(Dutta et al., 2008).***

Laboratory findings in hypothyroidism may include hyponatremia, hypercapnia, hypoxia, normocytic anemia, elevated creatine kinase, hyperprolactinemia, and hyperlipidemia ***(American Academy of Family Physicians, 2012).***

Table (1): Common symptoms of hypothyroidism

Arthralgias	Dry skin	Menorrhagia
Cold intolerance*	Fatigue*	Myalgias
Constipation	Hair thinning/ hair loss	Weakness
Depression	Memory impairment	Weight gain
Difficulty concentrating		

*—*Most common.*

Table (2): Clinical signs of hypothyroidism

Bradycardia	Laboratory results *(continued)*
Coarse facies	Increased creatine kinase
Cognitive impairment	Increased low-density lipoprotein cholesterol
Delayed relaxation phase of deep tendon reflexes	Increased triglycerides
Diastolic hypertension	Normocytic anemia
Edema	Proteinuria
Goiter	Lateral eyebrow thinning
Hypothermia	Low-voltage electrocardiography
Laboratory results	Macroglossia
Elevated C-reactive protein	Periorbital edema
Hyperprolactinemia	Pleural and pericardial effusion
Hyponatremia	

(American Thyroid Associatio, 2012)

<u>Screening and Diagnosis:-</u>

Family physicians should evaluate for thyroid dysfunction in all patients with symptoms of hypothyroidism. The American Academy of Family Physicians does not recommend screening for hypothyroidism in asymptomatic adults, ***(David et al., 2012)*** and the U.S. Preventive Services Task Force found insufficient evidence for routine screening in this population ***(Helfand, 2004).***

Screening of asymptomatic patients may be considered in those with risk factors for hypothyroidism, such as a history of autoimmune disease, history of head or neck irradiation,

previous radioactive iodine therapy, presence of a goiter, family history of thyroid disease, or treatment with drugs known to influence thyroid function. The best laboratory assessment of thyroid function, and the preferred test for diagnosing primary hypothyroidism, is a serum TSH test ***(Levy et al., 2012).***

If the serum TSH level is elevated, testing should be repeated with a serum free thyroxine (T4) measurement ***(Surks et al., 2004).***

Overt primary hypothyroidism is indicated with an elevated serum TSH level and a low serum free T4 level.

An elevated serum TSH level with a normal range serum free T4 level is consistent with subclinical hypothyroidism. A low serum free T4 level with a low, or inappropriately normal, serum TSH level is consistent with secondary hypothyroidism and will usually be associated with further evidence of hypothalamic-pituitary insufficiency. It is important to interpret these measurements within the context of the laboratory specific normative range for each test. Diurnal variations exist in TSH secretion such that the lowest level will generally be obtained with a morning laboratory draw ***(Roelfsema et al., 2009).***

Free T4 is usually measured by automated analog immunoassays. In most instances, this assay will yield accurate results. However, abnormal types or quantities of binding

proteins may be present in some patients and may interfere with the accurate measurement of free T4 by analog immunoassays. These problems can be overcome by measuring free T4 via equilibrium dialysis ***(Soldin et al., 2005).***

Family physicians will most commonly encounter patients with primary hypothyroidism. Secondary hypothyroidism is present in only 5 percent of cases ***(Carle et al., 2006).***

Autoimmunity of the thyroid gland results in a spectrum of thyroid diseases in patients, the patients commonly presents with goiter or thyroid dysfunction, Etiologically patients with autoimmune thyroiditis including Hashimoto thyroiditis (chronic autoimmune thyroiditis) are usually hypothyroid and less commonly patients are hyperthyroid ***(Cooper and Landenson, 2012).***

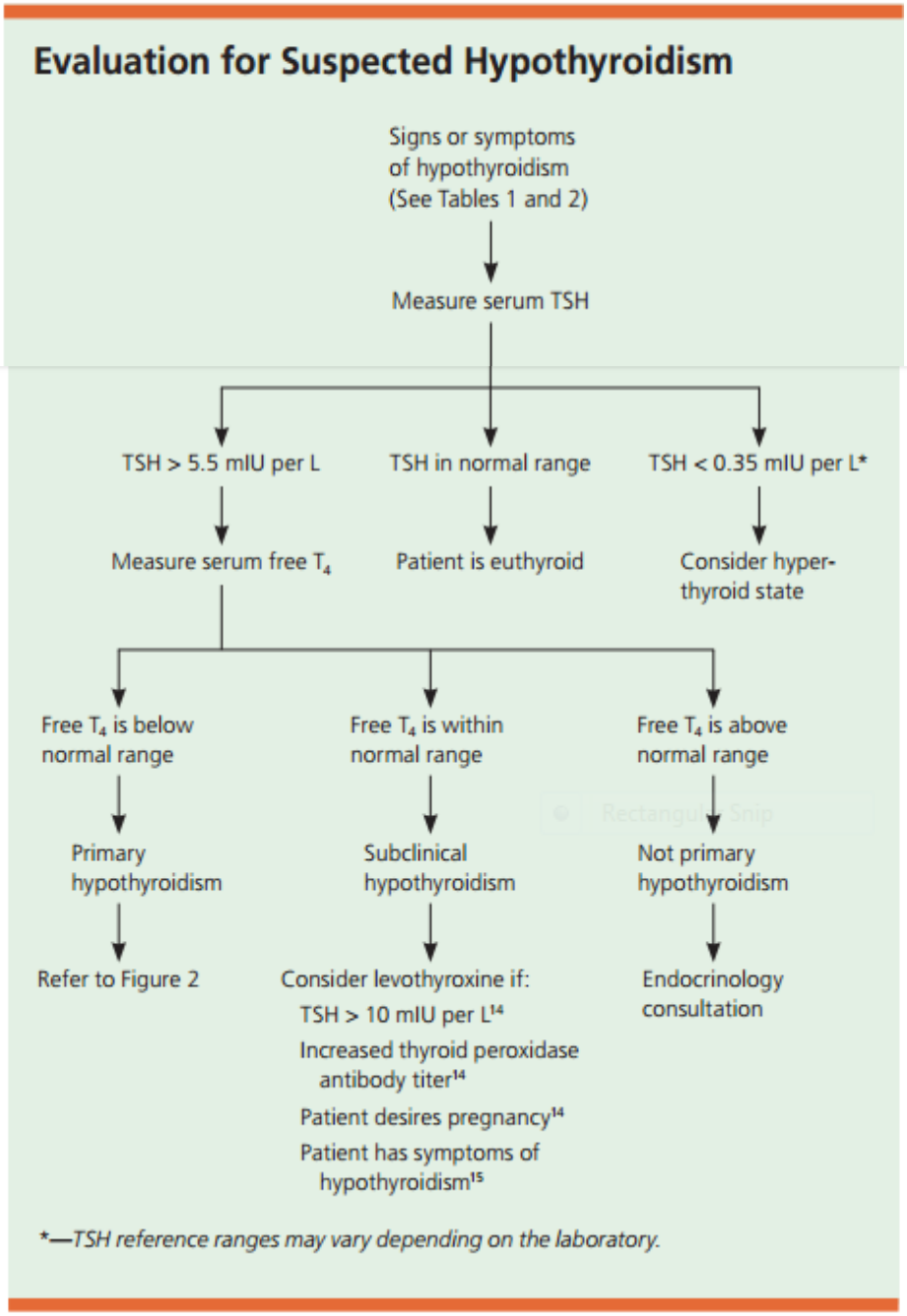

Figure (1): Algoristhm for evaluating suspected hypothyroidism (T_4=thyroxine; TSH= thyroid-stimulating hormone) ***(Levy et al., 2012).***

Hashimoto thyroiditis is an autoimmune, progressive inflammatory disorder of the thyroid gland. A dense lymphocytic infiltration of the gland is involved in the pathogenesis of Hashimoto thyroiditis. The incidence of this disease is 2% with a peak in women 30-50 years-old ***(Kirim et al., 2012).***

It is characterized by female preponderance, enlargement of the thyroid gland, thyroid autoantibody production and lymphocytic infiltration, associated with various degrees of thyroid hypofunction ***(Caturegli et al., 2014).***

Antithyroglobulin antibody (anti-Tg) and anti-thyroid peroxidase antibody (anti-TPO) are the main antibodies detected in Hashimoto thyroiditis. ***(Kirim et al., 2012).***

Thyroid peroxidase antibodies appeared to be much more prevalent than anti-thyroglobulin antibodies ***(McLachlan and Rapoport, 1992).***

Also Shinto and his colleagues found that thyroid peroxidase antibodies are more sensitive than anti-thyroglobulin antibodies in predicting hypothyroidism and in diagnosis of autoimmune thyroiditis ***(Shinto et al., 2010).***

Routine thyroid antibody estimation in patients withthyroid enlargement has revealed increase prevalence in autoimmune thyroiditis ***(Akkara, 2010).***

Many environmental factors associated with the development of autoimmune thyroid disease including stress, smoking, excess iodine, selenium deficiency, Vit D deficiency, irradiation, pollutants and infections ***(Weetman, 2011).***

Vit D is possibly more common in autoimmune hypothyroidism, Goswami and his colleagues studied the relation between Vit D (25 OH) and TPO and found that there is significant inverse association between Vit D (25 OH) level and thyroid autoimmunity as reflected by TPO (Abs) titers ***(Goswami et al., 2009).***

Also **Tamer and his colleagues** found that the prevalence of Vit D (25 OH) insufficiency in Hashimoto thyroiditis was significantly higher than that observed in healthy control and the prevalence rate of Vit D insufficiency showed a trend to be higher in patients with overt hypothyroidism or subclinical hypothyroidism than in those with euthyroidism ***(Tamer et al., 2011)***.

Although the exact mechanism of progressive thyroid tissue destruction is not clear, HT is regarded as a disorder of T cell-mediated immunity caused by an interaction between susceptibility genes, e.g., cytotoxic T-lymphocyte associated-4 (CTLA-4), human leukocyte antigen (HLA), TSH receptor (TSHR) and environmental factors, both of which are not yet completely understood ***(Lacka and Maciejewski, 2013).***

Measurements of serum concentration of TSH, total and free thyroxine (T4) and tri-iodothyronine (T3), antithyroid peroxidase (anti-TPO), antimicrosomal (anti-TM) or anti-thyroglobulin (anti-Tg) antibodies are more accurate than the enzyme-linked immunosorbent assay (ELISA) tests when performed with radioimmunoassays (RIA) or immuno-radiometric assays (IRMA). Indirect immunofluorescence (IF) tests, are also specific methods for the determination of autoantibodies against TPO and Tg and for HT ***(Zabek, 2002).***

Vitamin D also inhibits generation of cytokine which plays an important role in developing autoimmune thyroiditis ***(Deluca and Cantorna, 2001).***

Chapter 2

VITAMIN D

Introduction:-

Vitamin D (VD) is a group of fat-soluble pro-hormones which were identified after the discovery of the anti-rachitic effect of cod liver oil in the early part of the **20th** century ***(Zhang and Naughton, 2010)*.**

VD plays an important role in bone metabolism and seems to have some anti-inflammatory and immune-modulating properties**.** It has been observed that there are relationships between low **VD** levels and multiple disease states**.** Low **VD** levels are associated with increased overall and cardiovascular mortality**,** cancer incidence and mortality and autoimmune diseases such as multiple sclerosis ***(Kulie et al., 2009)*.**

Synthesis and Absorption:-

VD exists in two main forms**, VD3 (cholecalciferol)** and **VD2 (ergocalciferol),** differing in their side chain structure**.** ***In humans,*** the majority of **VD3** is produced in the skin from exposure to sunlight with a small proportion obtained from animal sources such as oily fish and egg yolk ***(Holick et al., 2011)*.**

VD2 is predominantly obtained from plant sources**.** Supplements of both **VD2 (produced from irradiation of the**