Hypovitaminosis D In Autoimmune Hypothyroidism

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Abstract: ***Objective:*** The present study investigates the total vitamin D (25 OH) Vit. D in 79 Egyptian autoimmune hypothyroid patients (AH) proved by assay of Thyroid peroxidase (TPO) Antibodies in their blood. ***Patients and methods:*** A 79 patients (65 females and 14 males) and a 14 apparently healthy individuals with matched age and sex were underwent a detailed clinical examination and routine laboratory tests in addition to thyroid function tests (TSH and FT4), Thyroid peroxidase (TPO) Antibodies and serum total Vit D (25 OH). **Result**: The patient group was classified according to the level of TSH into subclinical and overt hypothyroid groups. Levels of serum TSH were significantly increased in subclinical (X±SD 6.80±1.86 µIU/ml) and hypothyroid (X±SD 55.20±34.39 µIU/ml) groups as compared to control group (X±SD1.86±.99 µIU/ml) ($p< 0.001$). The TPO level was 414.73±435.73 IU/ml in subclinical hypothyroid group and was 1029.37±996.60 IU/ml in the hypothyroid group. The levels of serum total Vit D (25 OH) were significantly decreased in subclinical (X±SD 28.80±12.25 nmol/L) and hypothyroid groups (X±SD 11.57±3.70 nmol/L) as compared to control group (X±SD 90.86±12.60 nmol/L),($p< 0.001$). A highly significant negative correlation was found between serum TSH, TPO and total Vit D (25 OH) levels ($P < 0.001$). Also highly significant Positive correlation was found between the levels of serum total Vit D (25 OH) and serum FT4 ($P< 0.001$). There was significant Positive correlation between TSH and TPO levels ($P< 0.05$). **Conclusion:** Vit D (25 OH) deficiency is associated with AH and further studies are needed to determine whether its deficiency is the causal factor or the consequence of the disease.

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Introduction:

Autoimmune diseases are the third leading cause of morbidity and mortality in the industrialized world, surpassed only by cancer and heart diseases [(1)], despite this relatively high prevalence rate. The etiology and pathogenesis of most autoimmune disorders remain obscure and a number of factors have been implicated in their pathogenesis, one of the most recent agents found to be associated with autoimmunity is Vit D (25 OH).[(2)]

There is increasing interest in the role of Vit D deficiency in a number of chronic health problems including autoimmune diseases.[(3,4)] The demonstration of Vit D receptor in monocytes, dendritic cells and activated T cells indicate significant interaction between Vit D and immune system.[(5,6)] While the molecular mechanisms linking Vit D with autoimmunity are under investigations, in vitro studies indicate an immune-modulatory effect of 1,25 (OH) D on T helper1, T helper 2, T regulatory and dendritic cells leading to a shift towards activation of Thelper2. [(5,6)]

Vit D deficiency is increasingly recognized as a hidden health problem of uncertain but probably considerable importance and has been associated with several autoimmune disorders such as Type 1 DM, MS and rheumatoid arthritis. [(7)] Vitamin D has multiple immunosuppressant properties, supplementation of Vit D was shown to be therapeutically effective in various animal models such as encephalomyelitis [(8, 9)], and collagen induced arthritis [(10)], type 1 DM [(11)], inflammatory bowel disease [(12)] and autoimmune thyroiditis. [(13)]

Autoimmunity of the thyroid gland results in a spectrum of thyroid diseases in patients, the patients commonly present in outpatient endocrine clinics 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. [(14)] The clinical diagnosis of autoimmune thyroid diseases is usually confirmed by the detection of various antibodies in the patient serum, Three types of antibodies are most commonly assayed; Antithyroglobulin, Thyroid peroxidase (previously termed antimicrosomal) and TSH receptor antibodies. [(14)] Routine thyroid antibody estimation in patients with

thyroid enlargement has revealed increase prevalence in autoimmune thyroiditis. [15]

In recent years, the hypothesis has gained ground that entities likes puberty goiter in adolescents and multinodular goiter in adults are due to autoimmune thyroiditis, however these conditions were not previously diagnosed because tests for antibody estimation were not available.[15] Thyroid peroxidase antibodies appeared to be much more prevalent than antithyroglobulin antibodies.[16] Also Shinto, et al., (2010) [17] found that thyroid peroxidase antibodies are more sensitive than antithyroglobulin antibodies in predicting hypothyroidism and in diagnosis of autoimmune thyroiditis.

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.[18] Vit D is possibly more common in autoimmune hypothyroidism, and Goswami et al., (2009)[19] study 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. Also Tamer et al., (2011) [20] 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.

Aim of the work:

The aim of this work is to assess total Vit D (25 OH) (D2+D3) status in patients with autoimmune hypothyroidism proved by assay of TPO in those patients together with assessment of TSH and FT4.

Subject and methods:

This case control study was carried out at the outpatient clinic of endocrinology at Mansoura university hospitals between 1st of September 2012-30th of August 2013. A 93 subjects were involved In the present study, a 79 patients attending endocrinology outpatient complaining of symptoms of thyroid dysfunction with or without thyroid swelling (65 females and 14 males) and 14 apparently healthy individuals as a control group (11 females and 3 males). After taking a medical consent, A detailed history, full clinical examination were done to all subjects. 12 ml of venous blood was withdrawn from all subjects as follow: 3 ml in EDTA tube for complete blood picture and 9 ml in 3 Plain tubes for the rest of our workup, after clotting of blood in the plain tubes, centrifugation was done at 3000 rpm for 10 min. The collected serum was stored at -20 till the time of assay. Our work up include the following investigations: Routine investigation (FBG, Creatinine, T. cholesterol, TG, HDL, LDL and VLDL), Thyroid function tests (hypersensitive TSH and FT4), Thyroid peroxidase (TPO) Antibodies, and Total Vit D (25 OH). According to TSH levels the thyroid patients were subdivided into two groups according to TSH level into subclinical hypothyroid group (TSH <10 µIU/L) and overt thyroid group (TSH > 10 µIU/L).

Assay:

1-FBG,S.Creatinine and S. Lipids was carried out using a fully automated analyzer unicell DXC 800 Beckman coulter with intra-assay and inter-assay CV for these tests ranged between 3.5and 5.0 %.

2-Hypersensitive TSH (h TSH) was carried out using a one step-sandwich immunoassay tech. of a unicell DXI 600 Beckman coulter (USA),the analytical sensitivity is 0.003µIU/ml and the expected value from (0.34-5.6 µIU /ml). FT4 was carried out using a two step competitive immunoassay tech. of a unicell DXI 600 Beckman coulter (USA); the analytical sensitivity is 1.9 pmol/L and the expected value (7.5- 21.1 pmol/L).

3- TPO antibodies, was carried out using a chemiluminescence immunoassay of the unicell DXI 800 Beckman coulter and the expected value is (0- 75 IU/ml).

4-VIT D 25(OH),was carried out by the 2nd generation platform of ECL (Electrochemiluminescence) technology of Cobas 4111 Hitachi-Roche diagnostics GmbH d-68298 Mannheim Germany,and the reference range < 50 nmol/L deficient, 50-75 nmol/L insufficient,and > 75 nmol/L sufficient.

Statistical analysis:

SPSS package version 20 for Windows was used for the statistical analysis of data. Chisquare test and Kruskal Wallis test were done for non parametric variables. Spearman correlation coefficient was used to find out the association between TSH, FT4, VIT D and Thyroid auto antibodies (TPO).P value < 0.05 is considered significant.

Results:

Table (1) shows the age and sex matched P>0.05, not significant. The mean values of HB, Glucose, Creatinine and complete lipid profile tests show no significant statistical difference between groups (p> 0.05),while mean levels of TG show significant statistical difference between groups (P<0.05).

Table (1) demographic characteristics and basic laboratory results of the studied groups.

Characteristics	Thyroid disease group(n=97)		Control group (n=14)X±SD	P Value
	Subclinical Hypothyroidism (n=37) X±SD	Hypothyroidism (n=42) X±SD		
Age (year)	38.86±17.35	37.40 ±16.77	25.93 ±15.62	P>0.05
sex	Male 5(13.5%), female32(86.5)%	Male 9(21.4%), female33(78.6)%	Male 3(21.4%), female 11 (78.6)%	P>0.05
HB(g/l)	127.89±12.43	121.86±19.84	126.71±12.29	P>0.05
GLUCOSE (mmol/L)	6.28±3.11	6.02±2.56	5.99±2.91	P>0.05
CREATININE µmol/L)	64.59±24.09	113.90±173.99	65.57±43.12	P>0.05
CHOLESTEROL (mmol/L)	4.91±0.85	5.35±1.46	4.97±0.92	P>0.05
TG (mmol/L)	1.07±0.97	1.38±1.21	0.71±0.41	P<0.05
HDL(mmol/L)	1.37 ±0.35	1.38±0.39	1.51±0.54	P>0.05
LDL(mmol/L)	2.99±0.57	3.29±1.03	3.14±0.78	P>0.05
VLDL(mmol/L)	0.44±0.39	0.57±0.49	0.32±0.18	P>0.05

Table (2) the levels of serum TSH were significantly increased in subclinical and hypothyroid groups as compared to control group (p< 0.001). The levels of serum VIT D were significantly decreased in subclinical and hypothyroid groups as compared to control group (p< 0.001).

Table (2): Mean and standard deviation (X±SD) of thyroid function tests, TPO and VIT D in studied groups.

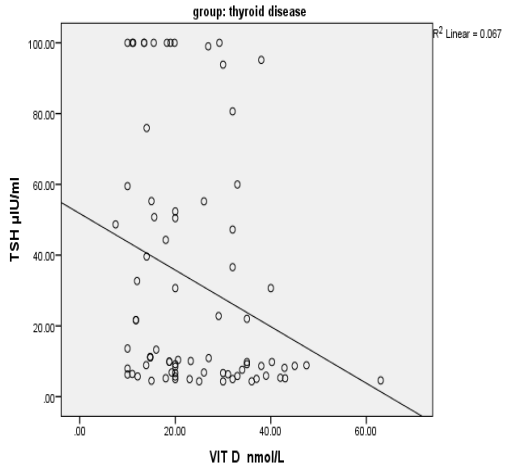
characteristics	Thyroid disease group(n=97)		Control group (n=14)X±SD	P Value
	Subclinical Hypothyroidism (n=37)X±SD	Hypothyroidism (n=42)X±SD		
TSH(µIU/ml)	6.81±1.86	55.20±34.39	1.86±0.99	P<0.001
FT4(PMOL/ML)	10.39±1.99	6.56±3.57	12.41±1.05	P<0.001
VITD(NMOL/L)	28.80±12.25	11.57±3.70	90.86±12.60	P<0.001
TPO (IU/ml)	414.74 ±435.74	1029.38±996.60	.0000	P<0.001

Tables (3) A highly significant negative correlation was found between serum TSH and Vit D levels P < 0.001 (Fig.1), also a highly significant negative correlation was found between serum Vit. D and TPO levels P < 0.001 (Fig.2).A highly significant Positive correlation was found between the levels of serum VIT D and serum FT4 levels P < 0.001(Fig 3).

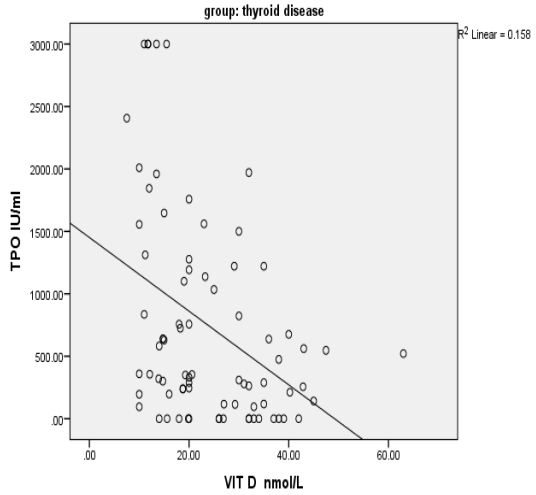
Table (3) Correlation coefficients' (r) between Vit D and thyroid function tests among auto immune hypothyroid group (no=79).

Thyroid function tests	SERUM VITAMIN D (r)	p
TSH	-0.626	P<0.001
FT4	0.454	P<0.001
TPO	-0.368	P<0.001

FIGER 1 SHOW CORRELATION BETWEEN TSH AND VIT D IN THYROID DISEASE GROUP



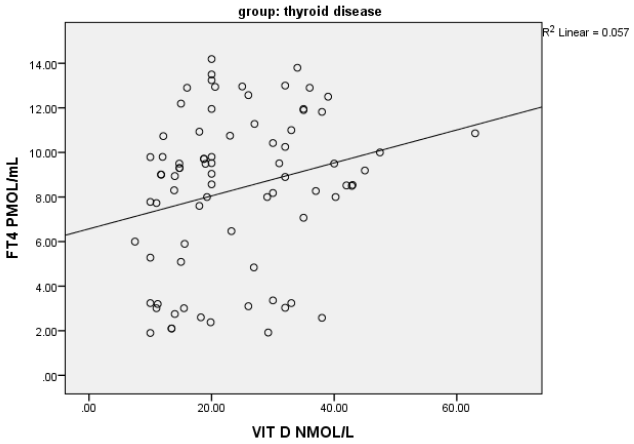
FIGER 2 SHOW CORRELATION BETWEEN VIT D AND TPO IN THYROID DISEASE GROUP



Discussion:

Besides bone mineral homeostasis, Vit D (25 OH) deficiency has been associated with a wide range of non-skeletal effects including predisposition towards autoimmune disorders.[13],and vitamin D supplementation resulted in decreased prevalence of autoimmune disorders such as type 1 diabetes and multiple sclerosis. A recent meta-analysis showed 29% reduction in the risk of type 1 diabetes in children receiving vitamin D supplementation [21,22]. Similarly, in multiple sclerosis every 50 nmol/l increase in serum (25 OH) Vit D levels in a healthy Caucasian population reduced the risk of disease by 41% [23].

FIGER 3 SHOW CORRELATION BETWEEN VIT D ANF FT4 IN THYROID DISEASE GROUP



Our study demonstrated that there was a highly significant decrease in (25-OH) Vit D levels in autoimmune hypothyroid patients both in the subclinical (X±SD 28.80±12.25 nmol/L) and hypothyroid groups (X±SD11.57±3.70 nmol/L) as compared to control group (X±SD 90.86±12.60 nmol/L) (p< 0.001). The autoimmunity of those patients was proved by assessment of TPO and the result of it was (X±SD 414.74±435.74 IU/ml) in subclinical hypothyroid group and (X±SD 1029.38±996.60 IU/ml) in the hypothyroid group where it was negative in the control group. On the same side Kivity et al.,2011 [5] found that The prevalence of vitamin D (25-OH) deficiency was significantly higher in patients with autoimmune thyroid diseases (AITDs) compared with healthy individuals (72% versus 30.6%; P<0.001) as well as in patients with Hashimoto's thyroiditis compared to patients with non-AITDs (79% versus 52%; P<0.05). We also found that there is a highly significant negative correlation between serum TSH, TPO And Vit D (25-OH) Levels P < 0.001 in both groups of patients of AH this means that vitamin D deficiency also correlated to the presence of antithyroid antibodies and abnormal thyroid function tests suggesting the involvement of vitamin D in the pathogenesis of AH. These results depend on the fact that vitamin D mediates its effect though binding to vitamin D receptor (VDR), and activation of VDR-responsive genes, while VDR gene polymorphism was found to be associated with autoimmune thyroid diseases.[5]

The activated VDR expresses CYP24, the enzyme primarily responsible for breaking 1,25-(OH) Vit D down into the inactive vitamin D metabolites. This exerts a feedback control on the maximum level that 1,25 -(OH) Vit D will attain.[24] However, CYP24 is suppressed in autoimmune disease, allowing 1,25-(OH) Vit D to reach unusually high

levels with low level of inactive form (25 –OH) Vit D [25]. Also Goswami et al., (2009)[19] found that there is significant inverse association between (25 OH) Vit D level and thyroid autoimmunity as reflected by TPO (Abs) titers.

On the other hand Effraimidis et.al.,2012 [26] found that early stages of thyroid autoimmunity are not associated with low vitamin D (25 OH) levels, and this is my be in early stage of the disease in which the level of autoantibody is not enough to affect level of CYP24, the enzyme primarily responsible for breaking 1,25-D down into the inactive vitamin D metabolites. Low levels of (25 OH) Vit D have been tied to a higher incidence of autoimmune disease, leading to the consensus that vitamin D "deficiency" may be a risk factor for autoimmune disease.[27]

However, the low levels of **(**25 OH) Vit D often observed in autoimmune disease must also be viewed in the light of data advanced by Marshall, 2008 in which low (25 OH) Vit D levels are the result of the autoimmune disease process rather than part of its cause.[24]. When active, transcription of CYP24 by the VDR keeps 1,25- (OH) VIT D levels in the normal range.[24] If the VDR is disabled by disease and unable to express CYP24 patients should display higher than normal levels of 1,25-(OH) VIT D. Studies on Crohn's disease, ulcerative colitis, RA, Sjogren's, and other autoimmune diseases confirm a higher than normal level of 1,25- (OH) VIT D among study subjects.[28] And Blaney 2008 reported that 1,25-(OH) VIT D levels were above the accepted range in the majority of his cohort of 100 patients with autoimmune disease with Low levels of (25 OH) Vit D.[29]

Recommendation:

Both (25 OH) Vit D and 1,25- (OH) VIT D must be measured in patients with autoimmune hypothyroidism as the presence of inhibited (25 OH) Vit D expression or excessive 1,25-(OH) VIT D expression both act as reliable markers of the disease process and are best interpreted in relation to one another and supplementation of (25 OH) Vit D is recommended.

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