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The Relationship between Serum Vitamin D Levels and Thyroid Functions in Patients with Hashimoto's Thyroiditis from Saudi Arabia

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AA presented the concept and idea of the research, managed the resources, edited the manuscript and supervised the project. AF and SH collected the data. SH and MIU carried out the experiment. AF analysed and interpreted the data. AA and AF validated the results. MIU and AF wrote the the manuscript with input from all authors.

Key words
Hashimoto's thyroiditis, Thyroid functions, Thyroid antibodies, Vitamin D, Ferritin

ABSTRACT

Hashimoto's thyroiditis (HT) is a chronic devastating autoimmune disorder of the thyroid gland, which develops due to environmental and genetic predispositions. Various studies have explored the role of 25(OH)-D deficiency in HT. Although vitamin D supplementation is suggested to improve thyroid functions, the mechanism of action of vitamin D on thyroid hormones remains unclear. The objective of the present study was to determine serum concentrations of thyroid hormones, serum thyroid antibodies, and serum 25(OH)-D and underscore the association of vitamin D with thyroid function in HT. A cross-sectional study comprising 150 subjects, including 80 HT cases and 70 healthy controls was carried out. The serum thyroid hormone concentrations (FT4, TSH), serum thyroid antibodies (anti-TPO, anti-Tg), serum 25(OH)-D, serum ferritin, blood hematocrit, and mean cell volume (MCV) were measured. Results showed that serum TSH, anti-Tg, and anti-TPO were significantly increased in HT cases than in the healthy controls, while serum FT4, 25(OH)-D and ferritin were significantly reduced ($p < 0.0001$). A negative correlation between serum vitamin D with anti-TPO and a positive correlation with FT4 and ferritin ($p < 0.05$) was observed. The results indicated that reduced serum levels of vitamin D are associated with higher serum anti-TPO and lower serum FT4 and ferritin levels in HT patients.

INTRODUCTION

Hashimoto's thyroiditis (HT) is a chronic thyroid autoimmune inflammation triggered by T cell-regulated $CD4^+$ cells, which influence the follicular cells of the thyroid gland. Autoimmune destruction leads to the infiltration of thyroid-specific B and T lymphocytes, which produce the thyroglobulin antibodies (TG-Ab) and thyroid peroxidase antibodies (TPO-Ab). This continuous immune destruction subsequently causes hypothyroidism and thyroid dysfunction in about 90% of the cases (Chahardoli *et al*., 2019; de Freitas *et al*., 2010). The frequency of HT is about 15 folds higher in females than males affecting the age groups between 30 and 50 years with apparent clinical presentation. It affects about 2% of the world population (Parvathaneni *et al*., 2012; Włochal *et al*., 2014), with a considerably higher incidence rate of 7-10% in the European population (Lossow *et al*., 2019). Studies conducted in different regions of Saudi Arabia indicate the prevalence of hypothyroidism between the ranges of 18.7% to 29.1% (Alanazi *et al*., 2018; Aljabri *et al*., 2019; Alqahtani, 2021) and a general prevalence of 47.34% in Saudi Arabia. However, there are no significant studies about the prevalence and biochemical indices of HT from Saudi Arabia. An understanding of the incidence of HT paralleled with suitable biochemical indicators can facilitate in streamlining the study of HT.

Clinically, HT is presented in two phenotypic forms, i.e. goiter and non-goiter (or atrophic) thyroiditis. In the goiter type of HT, the destruction of follicular cells occurs due to Th1 mediated cellular immunity. While the non-goiter type of HT is associated with Th2 mediated

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immunity that triggers the specific B-lymphocytes for the production of blocker stimulus of anti-thyroid stimulating hormone receptor antibodies (TRAb), causing the disease (Al-Alyani *et al*., 2018; Phenekos *et al*., 2004).

Vit. D (vitamin D) is a fat-soluble vitamin derived from sterols and has various biological properties (Holick, 2006), such as metabolic regulation, cellular growth, and differentiation (Orgiazzi, 2012). The active form of vit. D (calcitriol) modulates the immune system and targets different cells like B and T lymphocytes, monocytes, and macrophages. It also facilitates the differentiation of T-helper cells and cytokine (TNF-α, IL-4, and IL-17) secretions (Kim, 2017; Li *et al*., 2013; Zha *et al*., 2014).

Previous studies have reported the association of vit. D deficiency with thyroid autoimmunity. The hydroxylation of vit. D produces cholecalciferol, which binds to vit. D receptors (VDR), and exerts its immunomodulatory effect by regulating gene expression, especially in the immune cells. Thus, the deficiency of vit. D significantly impacts the development and progression of various autoimmune disorders like rheumatoid arthritis, type 1 diabetes mellitus, autoimmune thyroid diseases (AITDs), and multiple sclerosis (Varenna *et al*., 2012). Some reports also documented the association of low levels of vit. D with anti-TPO, subsequently triggering the apoptosis of B cells (Effraimidis and Wiersinga, 2014; Ma *et al*., 2015; Mazokopakis and Kotsiris, 2014; Unal *et al*., 2014).

The objective of the present study was to investigate a possible link between vit. D and thyroid functions by determining the serum concentrations of thyroid hormones, thyroid antibodies, and serum 25(OH)-D concentrations.

MATERIALS AND METHODS

Study population and sample collection

Before the start of this research, ethical approval was granted from the Local Ethical Board of Jouf University and Helsinki Declaration was applied to obtain the human individuals. A cross-sectional study comprising 150 subjects, including 80 diagnosed HT cases and 70 healthy controls, was carried out. HT patients having low FT4, high TSH levels, and high levels of anti-thyroid antibodies (anti-thyroid peroxidase antibodies and thyroglobulin antibodies) were included in the study. While the patients with thyroidectomy, acute diseases, steroid drugs, and vit. D supplements, infectious hepatitis, heart, liver, and advanced kidney diseases were excluded.

After written informed consent, 5.0 ml of blood was collected aseptically for biochemical analyses. The blood sample from each participant was transferred into serum separating vacutainer, and serum was obtained using the standard protocol. Serum samples were stored as aliquots at -20□ for further analysis. Samples were analysed for thyroid antibodies (TPO-Ab, TG-Ab), thyroid function test, and serum vit. D levels.

Measurement of thyroid function

Thyroid profile was assessed through serum concentrations of FT4 and thyroid-stimulating hormone (TSH) in HT cases and healthy controls. Supporting parameters for the baseline diagnosis for HT, i.e., serum levels of thyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibody (anti-Tg), were determined by ELISA method using Microplate Immunoassay reader (Human Elsys Quattro, USA).

Serum levels of anti-Tg and anti-TPO antibodies

Measurement of anti-thyroglobulin (anti-Tg) was done using the ELISA method. It is a semi-quantitative method for the detection of IgG autoantibodies against thyroglobulin antigen in human serum. The presence of Tg-Ab, anti-thyroid peroxidase antibodies (anti-TPO), and thyroid function test, i.e., FT4 and TSH, in addition to the clinical findings were used to establish the diagnosis of Hashimoto's thyroiditis.

Serum 25(OH)-D and serum ferritin levels

Determination of serum 25(OH)-D levels was carried out by ELISA method using a Roche COBAS 8000 automatic Elisa reader. The optical density was measured and values were recorded for each subject. Serum ferritin measurement was conducted by Immulite 2000 auto-analyzer.

Statistical analysis

The Statistical Package for the Social Sciences program (SSPS version 22.0: IBM Corporation, NY, USA) and Microsoft Excel (Microsoft Inc., USA) were used for the data analysis. Student's t-test and two-tailed Mann Whitney U-tests were used for between-group comparisons of the median values of the variables. Pearson's correlation and linear regression analysis were used to establish an association between two variables. For multiple pairwise comparisons, the dataset was adjusted with Bonferroni adjustments. For the assessment of baseline sample characteristics, means, percentages, and mean values (minimum-maximum) were calculated. All the results were considered to be significant at ($p < 0.05$) and a confidence interval of 95%.

RESULTS

A total of 150 subjects were investigated, comprising

of healthy controls and HT cases. Out of the total study population, 80 were HT patients, including 20 males (25%) with a mean age of 48.71±15.88 years, and 60 females (75%) with a mean age of 36.36 ±13.13 years. Healthy controls were 70 in number, which includes 24 males (34.3%) with a mean age of 36.90 ± 18.99, and 46 females (65.7%) with a mean age of 33.89 ± 10.48 (Table I).

Table I. Demographic characteristics (Mean± SD) of the study participants in the healthy control and Hashimoto thyroiditis (HT) groups.

Character-istics	Health controls (N = 70)	Hashimoto thyroiditis cases (N = 80)
Age (years)		
Male	36.90±18.99	48.71±15.87
Female	33.89±10.48	36.76±13.13
Gender		
Male	24 (34.3%)	20 (25%)
Female	46 (65.7%)	60 (75%)

In HT cases and healthy controls, different biochemical parameters were determined and compared. A statistically significant difference (p <0.001) was observed between the concentrations of FT4 and TSH, thyroid antibodies (anti-TPO and anti-Tg), serum vit. D and serum ferritin in healthy controls and HT cases. Serum TSH and the thyroid antibodies were higher in HT patients, while serum 25(OH)-D and other biochemical parameters were significantly lower in patients than controls. In addition, the hematocrit was significantly lower in HT patients (Table II). A comparison of all biochemical parameters assessed in this study was carried out between healthy controls and HT patients using Mann-Whitney U-test (Fig. 1A-D).

The results, as indicated by the observed mean values, demonstrate that the HT patients have elevated levels of anti-TPO (888.43±598.49 IU/mL), anti-Tg (236.34 ± 93.9 IU/mL), and TSH (16.26 ± 28.75 mIU/L) as compared to anti-TPO (18.25±15.49 IU/mL), anti-Tg (3.53±2.18 IU/mL) and TSH (1.99±1.01 mIU/L) in controls. While serum FT4 (10.80 ± 2.76 pmol/L), serum 25 (OH)-D (32.57 ±14.34 nmol/L), ferritin (20.90 ± 12.92 µg/L) and blood haematocrit (35.09 ± 5.08%) were significantly reduced in HT patients than the controls.

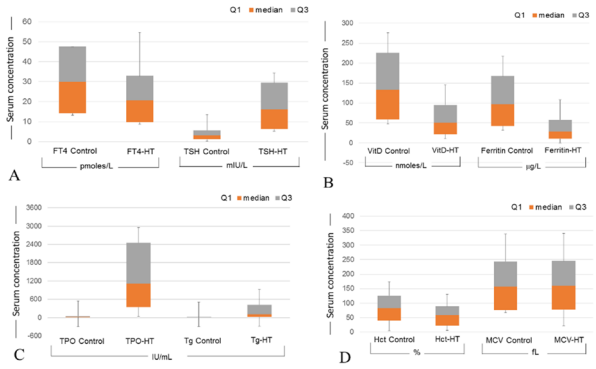

Fig. 1. Comparison of different biochemical parameters between the healthy control group (Control) and Hashimoto thyroiditis (HT) group. (A) Serum concentration of free T4 (FT4) and serum thyroid-stimulating hormone (TSH); (B) Serum concentration of 25(OH)D and ferritin; (C) serum anti-TPO (thyroperoxidase) and anti-Tg (thyroglobulin); (D) hematocrit (Hct) and Mean Corpuscular Volume (MCV) were tested in HT cases and in healthy controls. The x-axis represents the concentration of each biochemical parameter. The units for each biochemical parameter are displayed in the y-axis.

Table II. Association of nonparametric variables Cy in healthy control and HT groups.

Parameters	Healthy control (N= 70) Mean± SD	Hashimoto thyroiditis cases (N= 80) Mean± SD	p-value
Serum FT4 (pmol/L)	15.92 ±2.92	10.80 ± 2.76	< 0.0001*
Serum TSH (mIU/L)	1.99 ± 1.01	16.26 ± 28.75	< 0.0001*
Serum anti-TPO (IU/mL)	18.25 ± 15.49	888.43 ±598.49	< 0.0001*
Serum anti-Tg (IU/mL)	3.53 ± 2.18	236.34 ± 93.9	< 0.0001*
Serum 25(OH)-D (nmol/L)	78.37 ± 25.74	32.57 ±14.34	< 0.0001*
Serum Ferritin (µg/L)	64.40 ± 35.87	20.90 ± 12.92	< 0.0001*
Blood MCV (fL)	79.44 ± 11.87	80.31 ± 8.74	> 0.05
Blood Haematocrit (%)	42.08 ± 2.89	35.09 ± 5.08	< 0.05*

*p-value < 0.05 reported as statistically significant. Mann-Whitney U test was used to determine the association of categorical independent variables.

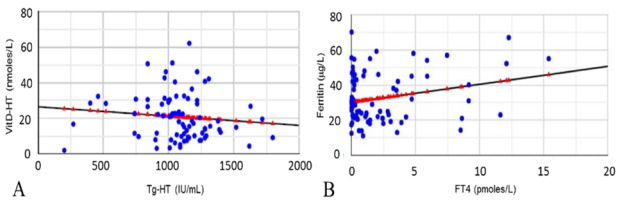

Fig. 2. Line fit plot for regression analysis. Regression analysis showed negative correlation (r = -0.221, p = 0.042) between (A) serum 25(OH)-D and anti-thyroglobulin (anti-Tg) and positive correlation (r = 0.240, p = 0.03159) between (B) serum FT4 concentration and serum ferritin levels.

Regression analysis indicated a negative correlation between serum vit. D and thyroglobulin antibodies (r = 0.221, p = 0.042) as observed by line fit plot (Fig. 2A**)**. Also, a positive correlation between serum FT4 and ferritin levels (r = 0.240, p = 0.03159) was observed (Fig. 2B).

DISCUSSION

HT is a predominant autoimmune thyroid defect that has no noteworthy management and treatment outcome thus far. It can progress into hypothyroidism and is supposed to become complicated as papillary cancer and thyroid lymphoma (Saraf *et al*., 2018). Global HT incidence has been increasing at an alarming pace, with tenfold higher prevalence than in the 1990s (Staii *et al*., 2010). Studies have attributed this increase in HT incidences to a simultaneous surge of vit. D deficiency or insufficiency worldwide (Chao *et al*., 2020; Mendes *et al*., 2020). Vit. D or 25(OH)-D is crucial for endocrine functions that are fundamental in maintaining homeostasis and regulation of bone and mineral metabolism. Deficiency of vit. D is linked to various diseases like autoimmune disorders, osteoporosis, adiposity, cardiovascular disease, infection, and cancer (Vilarrasa *et al*., 2010). Remarkably, vit. D has been studied for its significant immunomodulatory functions in the pathogenesis of various autoimmune diseases (Muscogiuri *et al*., 2015).

In the present study, we carried out a panel of biochemical analyses, which indicate that serum vit. D was significantly ($p < 0.0001$) lower in HT cases than the healthy controls. Serum TSH and antibodies (anti-TPO and anti-Tg) were significantly elevated, while serum FT4 was reduced in the patients than in the controls. Consistent with our findings, previous studies have also revealed that vit. D is frequently low in patients with HT (Bakr and Meawed, 2017; Chao *et al*., 2020) and negative association of anti-TPO with serum vit. D (Chahardoli *et al*., 2019). While, another study demonstrated that there was no association between anti-TPO and vit. D supplementation (Mazokopakis *et al*., 2015).

In present study, the majority of HT patients are asymptomatic, and that subclinical hypothyroidism may affect 8% of women and 3% of men (Farhangi *et al*., 2018). We found that TSH was significantly high ($p < 0.0001$) in the HT group, while compelling association was observed with reduced serum FT4 between groups, indicating that HT patients were more likely to have subclinical hypothyroidism. Furthermore, serum 25(OH)-D was significantly reduced in both the male and female patients with HT as compared to the control (Table III). Very few studies have been conducted to demonstrate the role of vit. D in the Saudi population. Though two studies showed that serum 25(OH)-D was reduced in the female thyroid disease patients than males, no association of Vit. D with thyroid disease was established (Al-Alyani *et al*., 2018; Bozkurt *et al*., 2013; Lippi *et al*., 2012; Mackawy *et al*., 2013).

In our study, we observed that TSH was higher in the HT group than in control. TSH is usually raised in response to low FT4, besides exerting its effect in the release of inflammatory factors. This signifies that TSH may be connected with the development of HT. TSH concentration was demonstrated to drop significantly in autoimmune thyroiditis patients upon supplementation with vit. D (Villa *et al*., 2020). On the other hand, sporadic reports also indicate that vit. D concentrations may not significantly affect the development of HT or other thyroid diseases. Some studies also describe a controversial relationship between HT and vit. D deficiency (Botelho *et al*., 2018; Zhao *et al*., 2021) or that the serum level of vit. D is not affected in the early phases of HT pathogenesis (Effraimidis *et al*., 2012). However, recent researches have provided compelling evidence towards the role of low concentrations of vit. D in the development of HT (Ma *et al*., 2015; Roehlen *et al*., 2018). Several meta-analysis reports describe the association of vit. D deficiency with the development of autoimmune thyroid disease (Štefanić and Tokić, 2020; Wang *et al*., 2015).

Previous investigations have also revealed a reverse correlation between serum concentrations of vit. D and TSH (Zhang *et al*., 2014), wherein high concentration of vit. D was associated with low serum TSH level, similar to the outcome of our study. Supplementation of vit. D was also demonstrated to decrease the thyroid antibodies titer, especially anti-TPO, suggesting that vit. D may be helpful in alleviating autoimmune diseases (Chahardoli *et al*., 2019; Krysiak *et al*., 2017).

Table III. Comparison of data on the gender variability in HT patients and controls.

	Female		Male	
	Control	**HT patient**	**Control**	**HT patient**
Free T4	15.44 ±2.01	10.82 ± 2.83**	16.85 ± 2.53	10.75± 2.61**
TSH	2.03 ± 0.97	17.78 ± 32.90**	1.93 ± 1.12	27.04 ±73.17**
TPO	20.61 ± 19.05	943.56 ±625.63**	17.35 ± 10.86	764.00 ± 300.03**
Tg	3.06 ± 2.71	405.08 ± 334.45**	6.05 ± 4.42	
Vitamin D	78.32 ± 25.83	32.60 ±17.13**	79.73 ± 29.36	32.03 ±12.87**
Ferritin	57.78 ± 29.91	19.41 ± 10.89**	79.35 ± 47.45	25.25± 16.98**
MCV	85.85 ± 4.05	79.01 ± 9.00*	88.68 ± 4.46	81.41 ±8.07**
Hematocrit	41.02 ± 2.19	33.91 ± 5.03*	44.12 ± 3.03	38.19 ±7.35**

*p-value < 0.05 reported as statistically significant. Mann-Whitney U test was used to determine the association of categorical independent variables. * P< 0.05; ** P <0.0001

We also determined serum ferritin concentrations, blood MCV, and hematocrit in HT patients and compared them with the healthy controls. Though blood MCV and hematocrit did not show a significant difference, we observed that serum ferritin was significantly reduced in HT cases. Low serum ferritin levels were previously demonstrated in HT patients (Ameen *et al*., 2019). Being an iron storage protein, ferritin releases its iron for a crucial cellular process. The enzyme thyroperoxidase requires iron for the synthesis of thyroid hormones. Low ferritin significantly impairs thyroid hormone synthesis and may lead to an increase in TSH levels in HT patients. Based on the results of this study, it is compelling to conjecture that reduced concentration of ferritin, significantly elevated levels of anti-TPO and anti-Tg, in conjunction with high TSH, may induce inflammatory cytokines production and decrease the concentration of antioxidants in the body. Estimation of serum ferritin concentration may help in understanding the etiology, pathogenesis, diagnosis, and monitoring of HT patients (Sahana, 2020).

Antibody-mediated thyroid gland destruction is caused by high TSH concentration, which induces dendritic cell maturation and, subsequently, CD4+ mediated Th2 response. Heightened Th2 response leads to the inflammation of the thyroid gland causing necrosis or apoptosis of the tissue. Sustained high TSH also facilitates the development of antibodies against the thyroid gland. Vit. D is represented to prevent excessive TSH secretion from the pituitary gland. This potentially blocks the Th2 response and generation of autoantibodies against the thyroid gland, thereby alleviating HT disease progression. Ferritin serves as an iron storage protein and provides iron for thyroperoxidase enzyme activity to synthesize T3 and T4 hormones. A decrease in either serum Vit. D or ferritin concentration seems to have a direct correlation with the progression of HT disease. Based on the results of our study and previous knowledge of HT disease, we have proposed a possible mechanism of HT disease pathogenicity (Fig. 3).

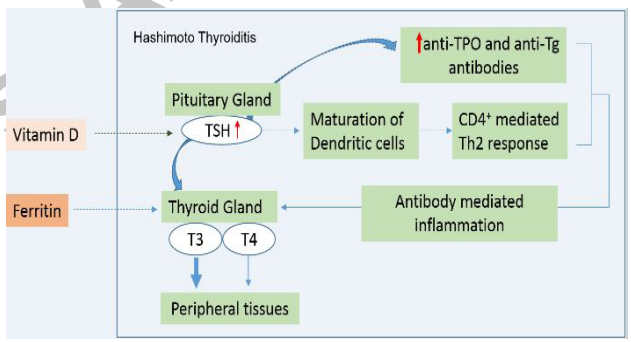

Fig. 3. Possible mechanisms of action of vit. D and ferritin in HT disease pathogenesis. Role of vit. D and ferritin is illustrated in the section and activation of hormones and thyroid antibodies.

CONCLUSION

We conclude that serum TSH, anti-TG, and anti-TPO were higher in HT cases while serum FT4, serum ferritin and haematocrit were significantly reduced in HT as compared to the control. A negative correlation between serum 25(OH)-D concentration and thyroglobulin antibodies and a positive correlation between serum ferritin and FT4 levels was found. On the other hand, reduced serum 25(OH)-D was associated with low levels of serum ferritin. Since vit. D modulates the Th1 towards Th2 response; maintaining an optimal level may help alleviated HT-associated inflammation. Subsequently, supporting evidences indicate that optimal vit. D concentration can also normalize TSH levels as well as decrease anti-TPO and anti-Tg antibody titres.

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Ethical Statement

Ethical permission for this study was taken from Local Ethical Board of Jouf University and Helsinki Declaration (modified 2013) was followed for human subject inclusion.

Statement of conflict of interest

The authors have declared no conflict of interest.

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