

## The Effect of Vitamin D Replacement on Subjects with Subclinical Hypothyroidism and Vitamin D Deficiency

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### Abstract

**Objectives:** There is a known inverse association between serum vitamin D level and Hashimotos thyroiditis from observational studies, however, very few interventional studies explored the effect of replacing vitamin D on the course of the disease. The aim of this study was to assess the effect of vitamin D replacement on serum thyroid autoimmunity, thyroid stimulating hormone (TSH), free T4 (FT4) in subjects with both autoimmune subclinical hypothyroidism (SCH) and Vitamin D deficiency.

**Methods:** We assessed the effect of vitamin D3 replacement on sixty-four Saudi patients with SCH (defined as TSH>4.2 but less than 10 mIU/L) and vitamin D deficiency (defined as plasma level<50, nmol/L). We measured TSH, FT4, anti TPO antibody titer (Anti TPO) and vitamin D levels at baseline and 3 months after supplementation of Cholecalciferol at a weekly oral dose of 50,000 IU.

**Results:** The mean age of the participants was 40.0 ± 1.8 years, mean body mass index was 29.2 ± 0.6 kg/m<sup>2</sup> and 64.7% of the participants were females. Mean TSH was 6.1 ± 0.3 mIU/L, mean FT4 was 14.5 ± 0.2 pmol/L, mean anti TPO level was 230.6 ± 96 IU/L and mean vitamin D level was 38.9 ± 3.6 nmol/L. Following 3 months of Cholecalciferol, serum 25 (OH) Vitamin D significantly increased to 62.1 ± 3.7 nmol/L, p<0.001, while TSH significantly decreased to 4.9 ± 0.26 mIU/L, p<0.001. There was a 64% reduction in anti TPO level which was negatively correlated with vitamin D level at baseline, r=-0.433, p=0.034.

**Conclusion:** Replacing vitamin D in Saudi subjects with mild autoimmune SCH and vitamin D deficiency significantly reduced serum TSH and improved thyroid autoimmunity. However, larger, randomized, controlled clinical trials for longer duration are needed to clarify the role of vitamin D replacement on the remission of mild SCH.

**Keywords:** Subclinical hypothyroidism; Vitamin D deficiency; Autoimmune thyroid disease

### Introduction

Vitamin D deficiency represents a global health problem and the prevalence of vitamin D deficiency (serum level<50nmol/L) in Saudi Arabia among different populations (adults, children and adolescents, newborns and pregnant/lactating women) has been shown to be about 80.0%, in line with most neighboring Gulf countries [1]. There are over one billion people worldwide with vitamin D deficiency or insufficiency [2]. Vitamin D deficiency is associated with osteoporosis and increased risk of falls & fractures [3]. Apart from its effect on bone metabolism, vitamin D has been shown to have immune modulatory and anti-inflammatory effects [4,5]. Moreover, several studies have linked vitamin D deficiency with autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), multiple sclerosis (MS) and Type 1 diabetes (T1DM) [6]. Furthermore, it has been reported that patients

with Hashimoto's thyroiditis had lower levels of vitamin D levels compared to healthy controls [7]. The association between low vitamin D levels and autoimmune thyroid diseases is supported by the recently published large meta-analysis that included more than 20 studies done in a large number of patients with Autoimmune Thyroid Diseases (AITD), namely Hashimotos Thyroiditis and Graves's Disease in comparison with controls in which Wang et al showed that patients with vitamin D deficiency had 2.99 odd ratio for having AITD (defined as either Hashimotos Thyroiditis or Graves' Disease) and 4.0 odd ratio for having Hashimotos Thyroiditis [8]. In addition, several studies described an association between AITD and polymorphisms of vitamin D Receptor (VDR), Vitamin D binding protein, and 25 hydroxylase or CYP2R1 [4,9]. Nonetheless, and despite the clear association between low vitamin D levels and AITD, very few interventional studies assessed the effect of vitamin D supplementation on thyroid autoimmunity and thyroid function tests [10,11]. Therefore, there is a need for more studies to explore this area. The aim of our study was to assess the effect of vitamin D supplementation on thyroid

autoimmunity and thyroid function test in Saudi patients with subclinical hypothyroidism and vitamin D deficiency.

## Methods

This study was a prospective, interventional study in subjects with subclinical hypothyroidism and vitamin D deficiency. A total number of 83 patients have been screened from patients attending the endocrine, diabetes, and family health clinics, if they had sub-clinical hypothyroidism (defined as serum TSH > 4.2 mIU/L but less than 10 mIU/L and FT4 in the normal reference range, 12-22 pmol/L) in addition to vitamin D deficiency (defined as serum 25 (OH) vitamin D level < 50 nmol/L). Serial thyroid function tests and vitamin D level measurements were done at baseline, and 3 months after taking 50,000 IU of Cholecalciferol weekly. The inclusion criteria were: adults aged 18-65 years with the diagnoses of subclinical hypothyroidism and vitamin D deficiency who were willing to sign informed consent and participate in the study. We excluded patients with known hepatic, renal or cardiac disease, type 1 diabetes, malignancy, pregnancy or lactation, malabsorption, inflammatory bowel diseases, metabolic bone disease, or patients taking medications that influence bone metabolism. Patients receiving thyroxine, amiodarone or lithium were also excluded from this study. All participants signed informed consent before participation in the study.

## Laboratory tests

Serum 25 (OH) D was measured with a Roche Elecsys modular analytics Cobas e411 using an electrochemiluminescence immunoassay (Roche Diagnostics, GmbH, Mannheim, Germany) and commercially available IDS kits (IDS Ltd, Boldon Colliery, Tyne & Wear, UK). Variation for the 25 (OH) D ELISA was 5.3% and 4.6%, respectively, with 100% cross-reactivity to 25 (OH) D3 and 75% cross-reactivity to 25 (OH) D2. It should be noted that the BRP laboratory is a participating entity in the Vitamin D External Quality Assessment Scheme (DEQAS), and Quality Assurance (QA) standards are maintained by ISO 9000 and 17025. Serum TSH and FT4 were estimated using commercially available kits by Roche Elecsys Modular Analytics Cobas e411 utilizing electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). TSH upper normal limit was 4.20 µIU/ml. Anti-thyroid peroxidase (TPO) antibodies (Ab) were also measured using commercially available ELISA kits (Bio-Line S.A, Brussels, Belgium) with a sensitivity of 1.4 U/ml (intra-assay variability 6.9%; inter-assay variability 13.4%).

## Statistical analysis

SPSS version 16 was used for statistical analysis. Paired Student t-test was used for comparisons between baseline variables and repeat measurements after 3 months. Statistical significance was set at  $p < 0.05$ . Data were presented as Mean  $\pm$  SEM. Pearson's co-efficient correlations were used for testing correlations.

## Results

Eighty-three Saudi patients were recruited for this study from the endocrine, diabetes, and family medicine clinics at King Fahad Medical City, Riyadh, Saudi Arabia. Sixty-eight of the subjects had complete baseline data and out of those, sixty-four patients completed the second visit after receiving vitamin D3 supplementation for 3 months. The baseline characteristics of the study population are shown in Table 1. The mean age of the study population was  $40 \pm 1.8$  years

old, mean weight was  $70.7 \pm 3.1$  kilograms, and 64.7% of the study population were females. The mean body mass index (BMI) of the study population was  $29.2 \pm 0.6$  kg/m<sup>2</sup>. The mean serum TSH was  $6.1 \pm 0.3$  mIU/L, mean free T4 was  $14.5 \pm 0.2$  pmol/L, and mean anti TPO anti-body titer was  $343.3 \pm 145$  IU/L. The mean serum 25 (OH) vitamin D level was  $38.9 \pm 3.6$  nmol/L and 80.7% of the initially screened study participants had vitamin D deficient state, defined as serum 25 (OH) vitamin D levels below 50 nmol/L (Figure 1). Table 2 shows the data of the sixty-four subjects who completed the study. Following 3 months of vitamin D replacement, serum 25 (OH) vitamin D level increased from  $33.2 \pm 3.2$  to  $62.1 \pm 3.7$  nmol/L,  $p$  value < 0.001. There was a significant reduction in TSH from  $6.0 \pm 0.23$  to  $4.9 \pm 0.26$  mIU/L,  $p$  value < 0.001, as shown in Figure 2. There was also a mild but significant increase in free T4 level from  $14.5 \pm 0.22$  to  $15.5 \pm 0.23$  pmol/L,  $p = 0.049$ . Serum Anti-TPO anti-body titer decreased by 64% from baseline (decreasing from  $343.4 \pm 145.9$  to  $125.1 \pm 58.2$  IU/L,  $p = 0.057$ ).

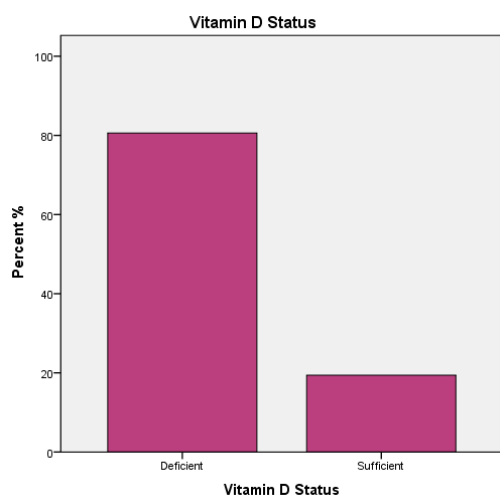
However, only twenty-nine subjects (forty percent) had 3 months follow up data (Figure 3). Using Pearson's co-efficient correlations, there was a strong negative correlation between baseline anti TPO antibody level and baseline vitamin D,  $r = -0.433$ ,  $P$  value was -0.034, however, there was no correlation between anti TPO antibody titer and either TSH or free T4. There was no statistically significant difference between baseline TSH in subjects with BMI > 30 kg/m<sup>2</sup> and < 30 kg/m<sup>2</sup>,  $6.2 \pm 0.36$  mIU/L and  $5.7 \pm 0.5$ , respectively,  $p = 0.55$ . Moreover, there was no correlation between baseline TSH and BMI.

Parameter	Mean $\pm$ SEM N=68	
Age (years)	40.0 $\pm$ 1.8	
Weight (kg)	70.7 $\pm$ 3.1	
Body Mass Index, (kg/m <sup>2</sup> )	29.2 $\pm$ 0.6	
Gender (male/female)	24/44	
Free T4 (pmol/L)	14.5 $\pm$ 0.2	12-22
Serum TSH level (mIU/L)	6.1 $\pm$ 0.3	0.24-4.2
Vitamin D level (nmol/L)	38.9 $\pm$ 3.6	<50: Deficient
		>50: Sufficient
Anti TPO antibody titre (IU/L)	230.6 $\pm$ 96.0	5>

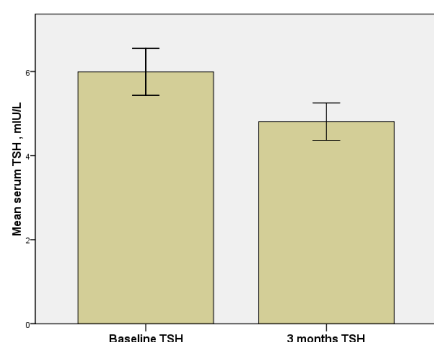
**Table 1:** Baseline characteristics of the study population.

Parameter	Baseline	Months 3	p Value
TSH mIU/L	6.0 $\pm$ 0.2	4.8 $\pm$ 0.2	<0.001
Free T4 Pmol/L	14.5 $\pm$ 0.22	15.0 $\pm$ 0.23	0.049
Vitamin D level nmol/L	33.2 $\pm$ 3.2	62.1 $\pm$ 3.7	<0.001
Anti TPO titre (N=29) IU/L	343.4 $\pm$ 145	125 $\pm$ 58	0.057

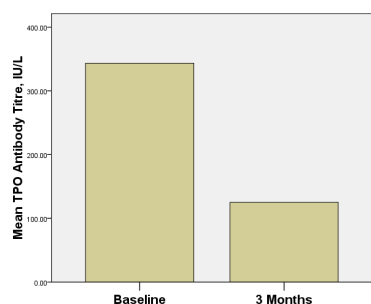
**Table 2:** The effects of Vitamin D replacement in the whole group of subjects with SCH and Vitamin D deficiency (N=64). Paired Student t-test,  $p < 0.05$  is significant.



**Figure 1:** Distribution of study population according to vitamin D deficiency, serum 25 (OH) vitamin D level<50 nmol/L and sufficiency (>50 nmol/L) status (N=68).



**Figure 2:** TSH level after 3 months of treatment with vitamin D in the study subjects (N=64). Paired t-test, p value<0.001.



**Figure 3:** Anti TPO antibody titer after 3 months of treatment with vitamin D in the study subjects (N=29). Paired t-test, p value<0.057.

## Discussion

Besides its beneficial effects on bone health and other target tissues, 1, 25 (OH)<sub>2</sub> vitamin D, is known to have multiple pleiotropic effects such as anti-inflammatory, immune-modulatory and anti-proliferative effects.

In the current study, we present very interesting results of an interventional, prospective study in which we examined the effect of vitamin D3 replacement on the course of mild autoimmune SCH in subjects with both mild SCH and vitamin D deficiency. We showed a significant improvement in some of the parameters of SCH, namely, a significant reduction in serum TSH, and a mild but significant increase in free T4, parallel with the rise in serum 25 (OH) vitamin D level following replacement.

Furthermore, anti TPO titers (as a marker of thyroid autoimmunity), decreased by 64% after three months although it did not reach statistical significance, however, the most likely explanation for this is the small sample size, as only 40% of the study population had anti TPO follow up data after three months.

Moreover, we reported a significant negative correlation between baseline serum 25 (OH) vitamin D level and baseline anti-TPO antibody titer, which has been previously reported and may support the role of vitamin D as an immune modulator, although the exact mechanism is not fully understood.

While several studies and one meta-analysis previously reported an inverse relationship between vitamin D level and autoimmune thyroid diseases [4,7,8], only very few studies examined the effect of replacing vitamin D on parameters of autoimmune sub-clinical hypothyroidism [10,11]. To our knowledge, our study is the first in Saudi Arabia and the third interventional study to examine the effect of replacing vitamin D on SCH parameters and thyroid autoimmunity [10,11].

In 2016, Chaudhary et al. published the first open-label randomized controlled trial that examined the effects of replacing vitamin D on patients with autoimmune thyroid disease and vitamin D deficiency that involved 46 subjects with SCH who received 60,000 IU of vitamin D and calcium for three months and a control group who received calcium supplements only [10]. In addition, Chaudhary et al. reported a significant reduction in anti TPO anti-body titer by 29% in the treatment group compared to 10% reduction in the control group and the difference between the two groups was significant at the end of the study with no significant changes in serum TSH and free T4 levels, unlike our study in which we showed, probably for the first time, significant changes in TSH and FT4 level following vitamin D supplementation. It is worth mentioning that Chaudhary et al. had a smaller number of participants (N=46 subjects) compared to our study which included 64 subjects. Another difference is that Chaudhary et al. defined the statistical significance with regards to the reduction in anti TPO titer by a reduction of more than 25% from the baseline, unlike our study in which we defined statistical significance by P value<0.05. It's interesting that Chaudhary et al. showed that the significant reduction in anti TPO and anti-thyroglobulin antibody titer was only observed in patients with TSH<10 compared to those with TSH>10 [10].

In another study by Simsek et al. from Turkey the authors reported a significant reduction in both anti TPO and anti-thyroglobulin antibody titers after vitamin D replacement in subjects with autoimmune thyroid diseases without significant changes in TSH and Free T4, however, this study differs from our study in a number of aspects [11].

First, both patients with Graves' disease and Hashimoto's Thyroiditis were included, which makes it incomparable to our study with regards to the interpretation of the changes in TSH and free T4 [11]. Other differences between our study and that was published by Simsek et al. is the dose of vitamin D used was only 1000 IU daily and for a duration of one month only with a number of subjects in the intervention group being only 36 subjects. Despite all the above-mentioned limitations, which all add to the strength of our study, Simsek et al. reported a significant reduction in anti TPO and anti-thyroglobulin antibodies levels in both patients with Graves's disease and Hashimoto's thyroiditis after vitamin D replacement, which again supports our findings regarding the reduction of thyroid autoimmunity following vitamin D replacement in these subjects.

Several studies reported the inverse association between low vitamin D level and thyroid autoimmunity [12-15]. However, the mechanism linking vitamin D deficiency to autoimmune thyroid disease is not fully understood and needs further exploration. Several authors suggested that the anti-inflammatory effect of vitamin D may lead to suppression of the immune system in vitamin D replete subjects [12-14]. Therefore, further mechanistic studies are needed to clarify the role of vitamin D deficiency in autoimmune thyroid diseases, especially if we take into account the very high prevalence of autoimmune hypothyroidism in Saudi Arabia, which has been estimated from previous studies to be around 43-47% [15,16].

We acknowledge several limitations to our study which first include the small sample size; however, this was mainly due to increased drop out of initially recruited participants due to different reasons including loss to follow up. Nonetheless, we believe our findings are still useful given the paucity of larger studies in this area. Secondly, the lack of a control group given the fact that the rate of spontaneous remission of SCH has been in the range between 4.0% to 37.5%, depending on the duration of follow up [15]. A third important limitation is the fact that we did not have data on the weight and BMI after 3 months of vitamin D supplementation given the fact that our population was mostly in the overweight to obese range and weight changes can affect the TSH level, however, although the initial TSH was slightly higher in the obese group, that difference was not statistically significant. Lastly, the lack of complete data on TPO antibody titers and lack of data on other thyroid antibodies such as anti-thyroglobulin antibody is yet another limitation.

## Conclusion

In conclusion, we report a very high prevalence of vitamin D deficiency in Saudi patients with SCH, reaching about 80%, which is in agreement with published literature regarding the association between low serum vitamin D level and AITD. More interestingly, we showed that replacing vitamin D3 for 3 months in these patients with mild autoimmune SCH and vitamin D deficiency significantly decreased their serum TSH level, mildly increased their FT4 level and markedly reduced their anti TPO titer by sixty-four percent, although this reduction did not reach to statistical significance, most likely due our small sample size. Our findings regarding the negative association between baseline vitamin D level and baseline anti TPO titer supports the hypothesis that autoimmune SCH may be linked to vitamin D deficiency; however, causality could not be concluded from this small study and further mechanistic studies are needed in this area. Moreover, there is a need for larger studies, ideally a randomized, controlled trial to confirm our findings.

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## Disclosures

None for all authors.

## Contributors

NA developed the idea of the study and was principal investigator. IB, MS, MSA, FA, FA, and SZ, SH, UE, AA, SA gathered and managed the data. IB also developed the statistical analysis plan and wrote the final report. All authors interpreted the data and contributed to revisions of the report.

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