Original Article

Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: An open-labeled randomized controlled trial

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ABSTRACT

Background and Aims: Although Vitamin D deficiency has been linked to autoimmune thyroid disorders (AITD), the impact of Vitamin D supplementation on thyroid autoimmunity is not known. This study aimed to evaluate the impact of Vitamin D supplementation on thyroid autoimmunity (thyroid peroxidase antibody [TPO-Ab] titers) in patients with newly diagnosed AITD in a randomized controlled trial. **Materials and Methods:** One hundred two patients with newly diagnosed AITD (TPO-Ab > 34 kIU/L and/or sonographic evidence of thyroiditis) patients were randomized into Group-1 (intervention group) and Group-2 (control group). Group-1 received cholecalciferol 60,000 IU weekly and calcium 500 mg/day for 8 weeks; Group-2 received calcium 500 mg/day for 8 weeks. Responders were defined as ≥25% fall in TPO-Ab titers. Individuals with at least 3-month follow-up were analyzed. Trial is registered at ctri.nic.in (CTRI/2015/04/005713). **Results:** Data from 100 AITD patients (68 with thyroid stimulating hormone [TSH] ≤10 mIU/L, 32 with TSH > 10 mIU/L), 93% having Vitamin D insufficiency, were analyzed. TPO-Ab titers were highest among patients in the lowest 25-hydroxyvitamin D quartile ($P = 0.084$). At 3 months follow-up, there was significant fall in TPO-Ab in Group-1 (−46.73%) as compared to Group-2 (−16.6%) ($P = 0.028$). Sixty-eight percentage patients in Group-1 were responders compared to 44% in Group-2 ($P = 0.015$). Kaplan–Meier analysis revealed significantly higher response rate in Group-1 ($P = 0.012$). Significantly greater reduction in TPO-Ab titers was observed in AITD with TSH ≤ 10 mIU/L compared to TSH > 10 mIU/L. Cox regression revealed Group-1 followed by TPO-Ab and free tetraiodothyronine levels to be a good predictor of response to therapy ($P = 0.042$, 0.069, and 0.074, respectively). **Conclusion:** Vitamin D supplementation in AITD may have a beneficial effect on autoimmunity as evidence by significant reductions in TPO-Ab titers.

Key words: Autoimmune hypothyroidism, autoimmunity, thyroid peroxidase antibody, thyroiditis, Vitamin D

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Introduction

Vitamin D insufficiency has been linked to cardiovascular disease, insulin resistance, fatty liver disease, type-2 diabetes

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and its complications, infections, and cancer.[1-4] It has also been linked to several autoimmune disorders including autoimmune thyroid disorders (AITD).[5-8] Observational studies have documented an inverse relation between serum levels of 25-hydroxyvitamin D (25[OH] D) and thyroid peroxidase antibody (TPO-Ab).[7-10] Cross-sectional nature and small sample size are major limitations of these reports. Data on the impact of Vitamin D supplementation on TPO-Ab in patients with AITD is not available. Hence, the aim of this present study was to evaluate the impact of Vitamin D supplementation on thyroid autoimmunity (TPO-Ab titers) in patients with newly diagnosed AITD in a randomized controlled trial (RCT).

Materials and Methods

Consecutive patients >18 years age, attending the endocrine clinic of the department, newly diagnosed with hypothyroidism within the last 3 months were considered. Patients with chronic kidney, liver or cardiac disease, malignancy, epilepsy, tuberculosis, immunodeficiency and those on chronic medications that might interfere with thyroid hormone or Vitamin D metabolism were excluded. Pregnant women and those who received Vitamin D and calcium supplementation in the last 6 months were also excluded. Patients started on levothyroxine supplementation at diagnosis were asked to continue levothyroxine. The patients were subjected to high-resolution ultrasonography (HRUSG) by a single operator blinded to the study design, using Toshiba Xario XG machine (Model number: SSA680A, Japan) with high-frequency transducer of 7.5–12 MHz. Thyroiditis was defined and graded as per the classification suggested by Raber *et al.* [Table 1].[11] AITD was defined as patients having serum TPO-Ab titer greater than the upper limit of normal for our laboratory (>34 kIU/L) and/or HRUSG evidence of thyroiditis. The study protocol was explained to the patients and those who gave informed written consent were included in the study. The study duration was from February 2013 to May 2015. The institutional ethics and research oversight committee approved the study protocol.

Table 1: Ultrasonography grading of autoimmune thyroid disorder

Grade number	Grade name	Ultrasonography features
1	Normal	Thyroid is hyperechoic to submandibular gland and prethyroid muscles (negative predictive value 96%)
2	Mild AITD	Thyroid is hypoechoic to submandibular gland but iso/hyper echoic compared to prethyroid muscles (sensitivity 84%)
3	Moderate AITD	Thyroid is hypoechoic to both submandibular and prethyroid muscles (sensitivity 58%; positive predictive value 95%)
4	Severe AITD	Thyroid is enlarged with severely hypoechoic compared to submandibular gland and prethyroid muscles

AITD: Autoimmune thyroid disorders

The included patients were called on a separate day after 12 h fast for clinical, biochemical, and anthropometric assessment. Height (to ± 0.1 cm) was measured in all the individuals using a Charder HM200PW wall-mounted stadiometer (calibrated using a 36" calibration rod [Perspective Enterprise, Portage, Michigan, USA]), and body weight (to ±100 g) measured using an electronic calibrated scale (Tanita, Japan, Model-HA521, Lot number-860525). Blood samples were collected; serum separated and stored at −80°C for estimation of free tetraiodothyronine (fT_4), thyroid-stimulating hormone (TSH), calcium, phosphate, and 25(OH) D. Fresh plasma extracted from the collected samples was transferred to the laboratory using cold chain for immediate estimation of intact parathyroid hormone (i-PTH).

Serum 25(OH) D was estimated using radioimmunoassay (Diasorin, Stillwater, MN, USA; analytical sensitivity 9.98 nmol/L; range 12.48–249.6 nmol/L; intra- and inter-assay coefficient of variation [CV] 4.5% and 11.3%, respectively). Estimation of fT_4, TSH, TPO-Ab, and i-PTH were performed using chemiluminescent microparticle immunoassay (Immulite-1000, Gwynedd, UK). Analytical sensitivity, range, intra- and inter-assay CV for the respective analytes were as follows: fT_4: 3.60 pmol/L (range 3.86–77.22 pmol/L), CV 4.1–9.8% and 5.2–12.1%, respectively; TSH: 0.004 mIU/L (range: 0.004–75 mIU/L), CV 4.5–13.8 and 8–17.5%, respectively; TPO-Ab: 7 kIU/L, range up to 1000 kIU/L, CV 3.5–5.6% and 7.8–10.5%, respectively; iPTH: 2 ng/L, CV 3.6–6% and 5.6–11.2%, respectively. Serum calcium and phosphate were estimated in all patients using clinical chemistry analyzer (Daytona, serial number-58260536, Furuno Electric, Nishnomeya, Japan).

The included patients were divided into two groups by a computer-generated randomization table, Group-1 (treatment group): AITD receiving Vitamin D supplementation, cholecalciferol 60,000 U (D-Rise sachets, USV, Mumbai, India) once weekly for 8 weeks along with tablet 1250 mg of calcium carbonate/day equivalent to elemental calcium 500 mg (Shelcal-500, Elder, Mumbai, India) and Group-2 (control group): AITD patients receiving tablet 1250 mg of calcium carbonate/day, for 8 weeks. This was an open-labeled RCT. The trial is registered with the Clinical Trial Registry of India at ctri.nic.in (CTRI/2015/04/005713). Subgroup analysis was performed by dividing AITD patients into two groups, Group-A: Patients having initial serum

TSH ≤ 10 mIU/L, and Group-B: Patients having initial serum TSH > 10 mIU/L.

Patients were followed up in the endocrine clinic monthly for the study duration. They were enquired about drug compliance, side effects, underwent examination, empty sachets and vials were collected, and fresh set of drugs issued. Patients were contacted telephonically/messaging services weekly to ensure compliance. Blood sampling was repeated after 3 months follow-up for all the biochemical parameters evaluated at baseline. Responders were arbitrarily defined as at least 25% fall in serum TPO-Ab titers. Individuals with at least 3-month follow-up were included for final analysis.

Statistical analysis

Normality of the distribution of variables was checked using the Kolmogrov–Smirnov test. Continuous variables were expressed as a mean ± standard deviation. $P < 0.05$ was considered as statistically significant. ANOVA with *post hoc* analysis and Kruskal–Wallis nonparametric ANOVA with Dunn's postcorrection were performed for normally and nonnormally distributed variables, respectively. Chi-squared tests were used for categorical variables. Pearson's or Spearman's correlation coefficient was calculated for normally and nonnormally distributed variables, respectively. Statistical Package for the Social Sciences (SPSS) version 16 (Chicago, Illinois, USA) was used for statistical analysis.

Results

Nine hundred and eighty-one consecutive patients of hypothyroidism were evaluated from which 102 AITD patients who fulfilled all inclusion and exclusion criteria and gave informed written consent were randomized into Groups 1 and 2. The study protocol and flow of patients have been elaborated in Figure 1. At least, 3-month follow-up data were available from 100 patients, which were analyzed. Sixty-eight AITD patients had baseline serum TSH ≤ 10 mIU/L (Group-A) and the remaining 32 patients had baseline serum TSH > 10 mIU/L (Group-B). None of the patients in Group-A were on levothyroxine supplementation. Of the 32 patients in Group-B, 12 had already initiated levothyroxine supplementation at the time of inclusion. Levothyroxine supplementation was initiated in the remaining 20 patients after inclusion into the study and randomization. Ninety-three percentage patients (93/100) in this study had Vitamin D insufficiency (25[OH] D < 75 nmol/L). Vitamin D deficiency (25[OH] D <50 nmol/L) was observed in 74% patients. All included patients had TPO-Ab titer >34 kIU/L. TPO-Ab titers were highest among AITD patients in the lowest

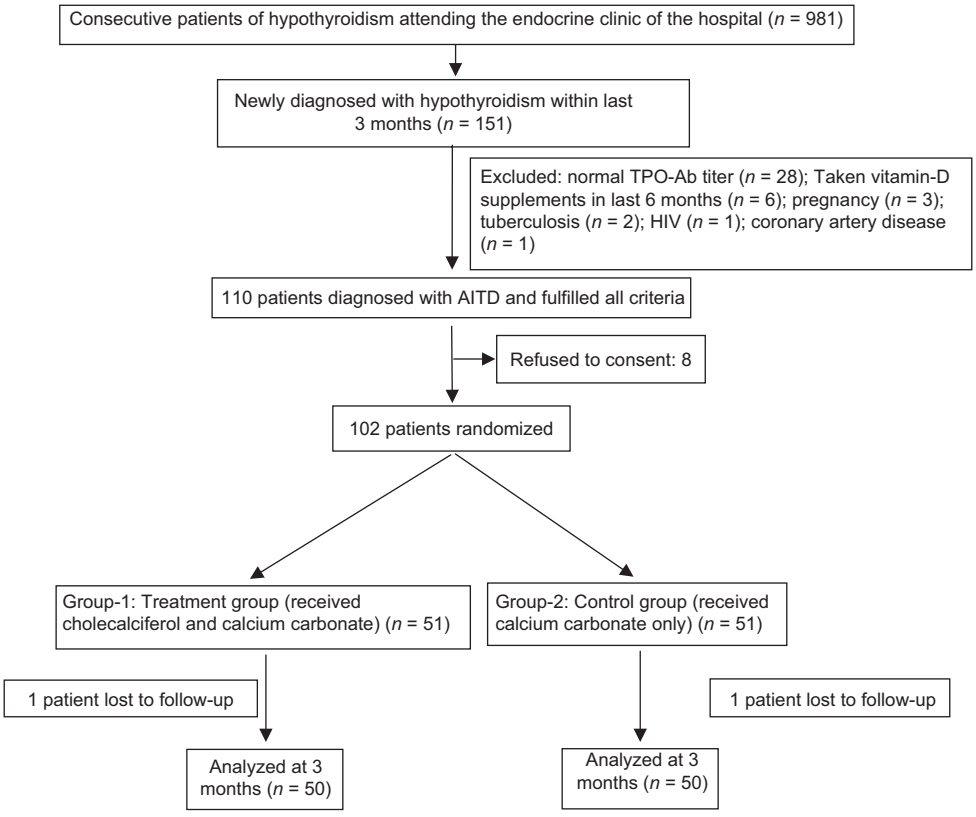

Figure 1: Flowchart elaborating the study protocol and flow of patients. AITD: Autoimmune thyroid disorder, TPO-Ab: Thyroid peroxidase antibody, HIV: Human immunodeficiency virus

25(OH)D quartile that approached statistical significance ($P = 0.084$) [Table 2]. HRUSG evidence of AITD was present in 92% of analyzed patients (92/100) [Table 2]. Ultrasonography features of mild (Grade-2) and moderate (Grade-3) AITD was observed in 48% and 44% of patients, respectively [Table 2 and Figure 2]. A negative correlation was observed between 25(OH) D and TPO-Ab titer, after adjusting for age, which approached statistical significance ($r = -0.184$; $P = 0.068$) [Table 3]. There were no adverse effects noted with Vitamin D and calcium supplementation. The compliance rate for Vitamin D sachets and calcium tablets were 99% and 96%, respectively.

Table 2: Baseline thyroid function, autoimmunity, calcium metabolism, and thyroid ultrasonography parameters as per quartiles of serum 25-hydroxyvitamin D

Parameter	Quartiles of Vitamin D (nmol/L) (*n*=100)				*P*
	1 (6.9-21.76) (*n*=25)	2 (21.76-36.24) (*n*=25)	3 (36.24-49.95) (*n*=24)	4 (49.95-124.18) (*n*=26)	
Age (years)	26.44±6.5	26.64±6.26	29.66±8.49	28.17±6.91	0.133
Sex (male:female)	4:21	6:19	6:18	8:18	0.672[a]
BMI (kg/m^2)	23.91±3.36	24±3.6	23.08±3.41	23.73±3.34	0.755
Free T_4 (pmol/L)	15.44±3.09	12.74±4.38	13.51±3.99	14.16±3.60	0.080
TSH (mIU/L)[b]	5.5 (118.7)	6.8 (149.43)	7.55 (73.57)	8.05 (99.98)	0.368[b]
TPO-Ab (kIU/L)	832.1±260.5	661±334.7	732.2±336.78	632.42±231.08	0.084
Calcium (mmol/L)	2.27±0.10	2.26±0.11	2.28±0.12	2.26±0.09	0.929
Phosphate (mmol/L)	1.27±0.21	1.24±0.18	1.30±0.17	1.24±0.17	0.664
iPTH (ng/L)	77.7±21.25	62.1±16.9	53.19±20.11	33.76±21.77	<0.001
Thyroid USG					
Normal (Grade-1)	0	4	3	1	-
Mild AITD (Grade-2)	11	12	11	14	0.905
Moderate AITD (Grade-3)	14	9	10	11	0.533

[a]*P* value calculated using Chi-square test; all continuous variables expressed as mean±SD; for continuous variables *P* value calculated using one-way ANOVA with *post hoc* analysis, [b]Variable not normally distributed expressed as median (range); Kruskal–Wallis nonparametric ANOVA with Dunn's postcorrection were performed; $P<0.05$ considered statistically significant. AITD: Autoimmune thyroid disease, BMI: Body mass index, T_4: Tetraiodothyronine, TSH: Thyroid stimulating hormone; TPO-Ab: Thyroid peroxidase antibody, iPTH: Intact parathyroid hormone, USG: Ultrasonography, SD: Standard deviation

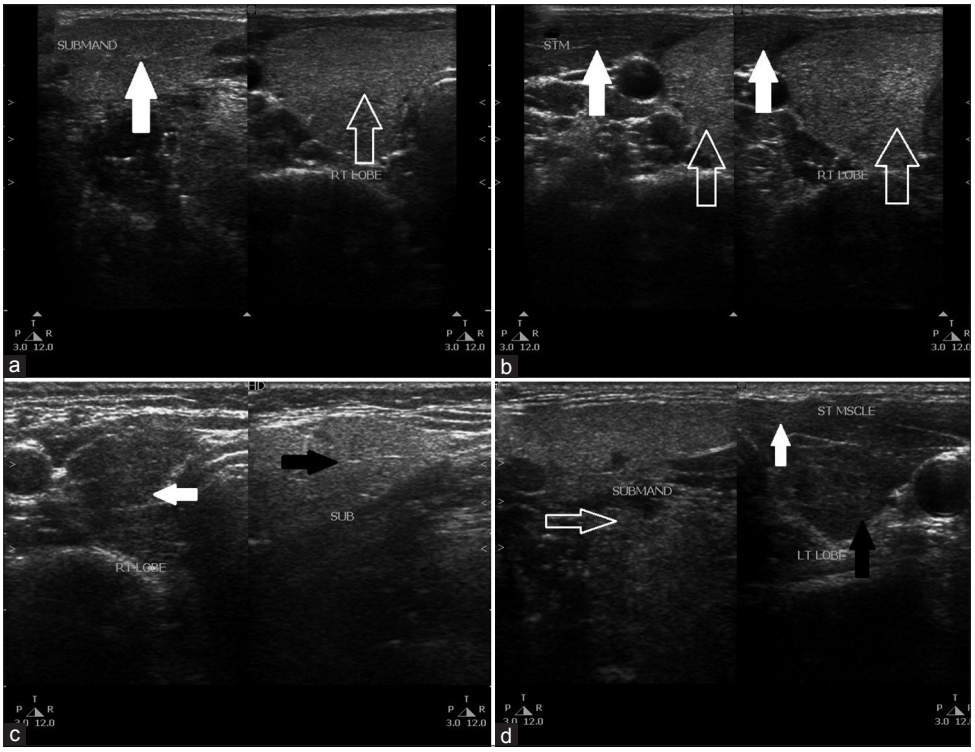

Figure 2: (a) Ultrasonography showing hyperechoic right lobe of thyroid (hollow white arrow) as compared to submandibular gland (solid white arrow) (normal, Grade-1). (b) Ultrasonography showing hyperechoic right lobe of thyroid (hollow white arrows) as compared to prethyroid muscles (solid white arrows) (normal, Grade-1). (c) Ultrasonography showing hypoechoic right lobe of thyroid (white arrow) as compared to submandibular gland (black arrow) (mild AITD, Grade-2). (d) Ultrasonography showing hypoechoic left lobe of thyroid (black arrow) as compared to both submandibular gland (hollow white arrow) and prethyroid muscles (solid white arrow) (moderate AITD, Grade-3). AITD: Autoimmune thyroid disease

The baseline age, anthropometry, thyroid function, and 25(OH) D levels were comparable in patients in Group-1 (treatment group) and 2 (control group) [Table 4]. At 3 months of follow-up, there was a significant fall in serum TPO-Ab titers in patients of the treatment group compared to controls [Table 4]. Median percentage change in TPO-Ab titer was −46.73% in Group-1 and −16.6% in Group-2 (P = 0.028). Greater than 25% reduction in TPO-Ab titer (responder rate) was achieved in 68% patients in Group-1 (treatment group) and 44% in Group-2 (control group) (P = 0.015). This evaluation achieved 79% statistical power, performing a one-sided test, using the current sample size of 50 patients each in both the groups, with 5% type-I error (alpha risk). Kaplan–Meier analysis showed that responder rate was significantly higher in Group-1 (treatment group) as compared to Group-2 (control group) (P = 0.012; Figure 3). Vitamin D supplementation led to a significant increase in serum 25(OH) D titers with a corresponding fall in plasma iPTH levels in Group-1.

Table 3: Correlation between thyroid hormone and calcium metabolism parameters after adjusting for age

Parameter	BMI	25(OH)D	iPTH
Free T_4	0.10 (0.365)	−0.06 (0.589)	−0.02 (0.848)
TSH	−0.08 (0.444)	0.12 (0.239)	−0.16 (0.109)
TPO-Ab	0.01 (0.927)	−0.184 (0.068)	0.10 (0.341)

All values are represented as Pearson's correlation coefficient followed by P value in parenthesis. BMI: Body mass index, 25(OH)D: 25-hydroxyvitamin D, iPTH: Intact parathyroid hormone, TPO-Ab: Thyroid peroxidase antibody, TSH: Thyroid stimulating hormone, T_4: Tetraiodothyronine

Table 4: Comparison of baseline and 3-month posttreatment thyroid function and autoimmunity parameters in patients of Group-1 (treatment group) as compared to Group-2 (control group)

Parameter	Group-1 (n=50)	Group-2 (n=50)	P
Age (years)	28.48±6.57	27.86±7.29	0.656
Sex (male:female)	11:39	13:37	0.640[a]
Smoking (smoker/ ex-smoker/nonsmoker)	0/1/49	0/1/49	1.000
BMI (kg/m^2)	24.03±3.7	23.42±2.94	0.371
Free T_4 (pmol/L)	13.90±3.86	14.03±3.99	0.866
TSH (mIU/L)[b]	6.88 (138.98)	6.8 (149.36)	0.783[b]
TPO-Ab (kIU/L)	739.1±343.2	687.8±255.1	0.398
Calcium (mmol/L)	2.25±0.09	2.28±0.12	0.261
Phosphate (mmol/L)	1.27±0.18	1.25±0.18	0.471
25(OH)D (nmol/L)[b]	33.25 (93.77)[b]	39.61 (116.31)[b]	0.391[b]
iPTH (ng/L)	62.13±22.86	50.91±27.08	0.027
Thyroid USG			
Normal (Grade-1)	6	2	0.140
Mild AITD (Grade-2)	22	26	0.423
Moderate AITD (Grade-3)	22	22	1.000
Levothyroxine dose (mcg)	83.79±22.78	84.56±14.70	0.841
3 months free T_4 (pmol/L)	16.47±2.06	16.86±1.93	0.468
3 months TSH (mIU/L)	3.16±2.07	3.39±2.19	0.605
3 months TPO-Ab (kIU/L)[b]	387 (1146)	553.5 (1002)	0.029
Percentage change in TPO-Ab (%)[b]	−46.73 (324.82)[b]	−16.6 (201.41)[b]	0.028[b]
3 months 25(OH)D (nmol/L)[b]	98.52 (124)[b]	41.61 (100.06)[b]	<0.001[b]
3 months iPTH (ng/L)[b]	19.1 (61.38)[b]	48.9 (87.14)b	<0.001[b]
Follow-up duration (months)	4.24±1.34	4.7±1.73	0.119
Responders (%)	34 (68)	22 (44)	0.015[a]

[a]P value calculated using Chi-square test; all continuous variables expressed as mean±SD; for continuous variables P value calculated using unpaired t-test, [b]Not normally distributed, represented as median (range), P value calculated using Mann–Whitney U-test; P<0.05 considered statistically significant. AITD: Autoimmune thyroid disorder, BMI: Body mass index, T_4: Tetraiodothyronine, TSH: Thyroid stimulating hormone, TPO-Ab: Thyroid peroxidase antibody, iPTH: Intact parathyroid hormone, USG: Ultrasonography, 25(OH)D: 25-hydroxyvitamin D, SD: Standard deviation

Sub-group analysis based on initial TSH revealed that Vitamin D supplementation in patients with baseline TSH ≤ 10 mIU/L (Group-A1) was associated with a significantly greater quantum of reduction in TPO-Ab titers with regards to the controls (Group-A2) (−29.06 vs. −10.96; P = 0.029) [Table 5]. In contrast, Vitamin D supplementation did not result in a significantly greater reduction in TPO-Ab titers with regards to the controls in patients with baseline TSH > 10 mIU/L (Group-B1 vs. Group-B2; −56.85 vs. −26.82, respectively; P = 0.077) [Table 5]. Cox regression showed that Vitamin D supplementation (Group-1) was an independent predictor of reduction in TPO-Ab titer by 25% (P = 0.042) [Table 6]. Baseline TPO-Ab titers and fT_4 levels were also good predictors of reduction in TPO-Ab titer, which approached statistical significance (P = 0.069 and 0.074, respectively; Table 6).

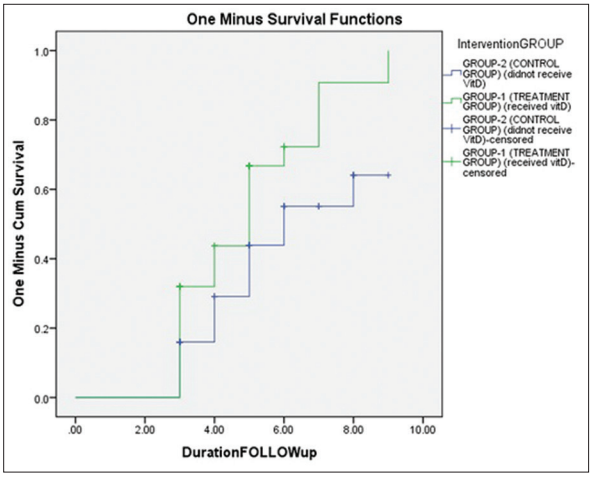

Figure 3: Kaplan–Meier analysis comparing responder rate among autoimmune thyroid disorder patients receiving Vitamin D and calcium supplementation (Group-1; treatment group) as compared to those receiving only calcium supplementation (Group-2, control group). Duration of follow-up in months; P = 0.012 (log-rank test [Mantel-Cox]). Group-1 (treatment group) AITD receiving Vitamin D supplementation, cholecalciferol 60,000 U once weekly for 8 weeks along with tablet 1250 mg of calcium carbonate/day equivalent to elemental calcium 500 mg and; Group-2 (control group) AITD patients receiving tablet 1250 mg of calcium carbonate/day, for 8 weeks. Responder was defined as at least 25% reduction in antithyroid peroxidase antibody titer from baseline. AITD: Autoimmune thyroid disease

Table 5: Comparison of baseline and 3-month posttreatment thyroid function and autoimmunity parameters in patients with baseline thyroid stimulating hormone ≤10 mIU/L (Group-A) as compared to those with baseline thyroid stimulating hormone >10 mIU/L (Group-B), as per the intervention group they were randomized into (Group-1 vs. Group-2)

Parameter	Group-A (*n*=68) TSH ≤10 mIU/L			Group-B (*n*=32) TSH >10 mIU/L		
	Group-A1 (*n*=34)	Group-A2 (*n*=34)	*P*	Group-B1 (*n*=16)	Group-B2 (*n*=16)	*P*
Age (years)	28.11±6.76	28.7±6.83	0.709	29.25±6.28	26±8.09	0.214
Sex (male:female)[a]	6:28	10:24	0.252	5:11	3:13	0.414
BMI (kg/m^2)	23.7±3.5	24.03±2.65	0.667	24.7±4.11	22.15±3.2	0.060
Free T_4 (pmol/L)	15.70±2.96	15.83±2.96	0.874	9.91±2.19	10.04±2.57	0.848
TSH (mIU/L)[b]	4.79 (9.98)	4.88 (9.16)	0.904[b]	52.11 (126)	56.8 (139)	0.723[b]
TPO-Ab (kIU/L)	722.8±336.6	704.32±251.3	0.257	786.18±350.45	652.68±267.67	0.235
Calcium (mmol/L)	2.25±0.09	2.29±0.12	0.145	2.25±0.09	2.25±0.11	0.846
Phosphate (mmol/L)	1.28±0.19	1.24±0.19	0.417	1.27±0.17	1.27±0.16	0.978
25(OH)D (nmol/L)[b]	36.62 (93.77)	36.72 (116.31)	0.987[b]	38.11 (58.51)	50.40 (72.38)	0.080[b]
iPTH (ng/L)	65.86±25.1	53.86±23.1	0.064	54.2±14.7	45.06±24.35	0.208
Thyroid USG[a]						
Normal (Grade-1)	5	1	0.087	1	1	1
Mild AITD (Grade-2)	14	15	0.806	8	11	0.280
Moderate AITD (Grade-3)	15	18	0.466	7	4	0.264
Levothyroxine dose (mcg)	-	-	-	86.75±23.48	87.62±15.08	0.901
3 months free T_4 (pmol/L)	16.43±2.19	16.73±1.67	0.592	16.34±1.8	16.86±2.45	0.632
3 months TSH (mIU/L)	2.98±1.63	3.49±1.84	0.602	4.10±2.60	4.39±2.04	0.789
3 months TPO-Ab (kIU/L)[b]	421 (1127)	579.5 (1002)	0.467[b]	258.5 (864)	505 (825)	0.289[b]
Percentage change in TPO-Ab (%)[b]	−29.06 (144)	−10.96 (201)	0.029[b]	−56.85 (194)	−26.82 (95)	0.077[b]
3 months 25(OH)D (nmol/L)[b]	97.54 (119.11)	40.24 (100.06)	<0.001[b]	102.71 (101.18)	55.51 (77.68)	<0.001[b]
3 months iPTH (ng/L)[b]	25.12 (61.38)	54 (87.14)	<0.001[b]	19.67 (37.64)	35.27 (56.48)	0.007[b]
Follow-up duration (months)	4.73±1.33	5.32±1.7	0.629	3.18±0.54	3.37±0.81	0.429
Responders[a]	22	13	0.028	12	9	0.264

[a]*P* value calculated using Chi-square test; all continuous variables expressed as mean±SD; for continuous variables *P* value calculated using unpaired *t*-test, [b]Not normally distributed, represented as median (range), *P* value calculated using Mann–Whitney U-test, *P*<0.05 considered statistically significant. AITD: Autoimmune thyroid disorder, BMI: Body mass index, T_4: Tetraiodothyronine, TSH: Thyroid stimulating hormone, TPO-Ab: Thyroid peroxidase antibody, iPTH: Intact parathyroid hormone, USG: Ultrasonography, 25(OH)D: 25-hydroxyvitamin D

Table 6: Cox regression showing variables that independently predict reduction in anti-thyroid peroxidase antibody titer by at least 25% (responder)

Variable	B	Exp(B)	*P*
Age	0.021	1.021	0.303
Sex	−0.058	0.944	0.867
BMI	−0.044	0.957	0.356
TPO-Ab titer	0.001	1.001	0.069
Free T_4	−1.178	0.308	0.074
TSH	0.009	1.009	0.120
25(OH)D	0.011	1.011	0.487
Intervention group (Group-1 vs. Group-2)	0.588	1.800	0.042
Dose of levothyroxine	0.006	1.006	0.513

Cox regression was done with baseline parameters age, sex, BMI, 25(OH)D, free T_4, TSH, intervention group allocated (Group-1 vs. Group-2) and dose of levothyroxine used to evaluate their role in the development of primary endpoint (responder), viz. at least 25% reduction in TPO-Ab titer; Exp(B): Exponentiation of the B coefficient, change in odds ratio with 1 unit change in predictor variable; for categorical variables sex and intervention Group, females and Group-2 (controls) were taken as reference group. BMI: Body mass index, TPO-Ab: Thyroid peroxidase antibody, T_4: Tetraiodothyronine, TSH: Thyroid stimulating hormone, 25(OH)D: 25-hydroxyvitamin D

Discussion

Vitamin D insufficiency has been linked to several autoimmune disorders including rheumatoid arthritis, lupus, type-1 diabetes, Crohn's disease, ulcerative colitis, and multiple sclerosis.[5-8] We have previously reported 74% prevalence of Vitamin D insufficiency in normal individuals, from Eastern India.[1,2] The prevalence of Vitamin D insufficiency among patients with AITD in this study (93%) is significantly higher and is in accordance with previous reports in patients with Hashimoto's thyroiditis and Graves' disease.[9,10] A recent cross-sectional study from Korea documented increased prevalence of TPO-Ab positivity along with ultrasonography features of AITD in premenopausal women with Vitamin D deficiency and insufficiency as compared to Vitamin D sufficient individuals.[12] A study from Thailand observed increased prevalence of Vitamin D deficiency and insufficiency in TPO-Ab or thyroglobulin antibody positive individuals when compared to those with negative antibody status.[13] In contrast, a cross-sectional population-based study from China did not observe any relation with Vitamin D status and TPO-Ab positivity. However, in that study an inverse relation was observed between 25(OH)D and TSH.[14] Early stages of thyroid autoimmunity (TPO-Ab positivity with normal serum TSH) were not associated with Vitamin D deficiency in a study from Holland.[15] A recent study from China reported an inverse correlation between serum 25(OH)D and TSH receptor antibodies in patients with Graves' disease, highlighting again the

link between Vitamin D and thyroid autoimmunity.[16] Different ethnicity, age, disease heterogeneity, and varying severity may explain the conflicting outcomes in different cross-sectional studies, warranting the need for interventional studies.

The only cross-sectional study from India has observed a weak inverse relation between TPO-Ab and Vitamin D status.[17] In our study also, there was a weak inverse relationship between TPO-Ab and 25(OH)D levels, which approached statistical significance. This is the first study to demonstrate that Vitamin D supplementation in therapeutic doses is associated with a significant reduction in TPO-Ab titers in patients with AITD. Subgroup analysis revealed that the quantum of reduction in TPO-Ab titers (with regards to baseline) in treatment versus control groups was significant only in patients with baseline serum TSH ≤ 10 mIU/L. This may be explained by a more advanced disease state in patients with baseline TSH > 10 mIU/L. Hence, Vitamin D supplementation in newly diagnosed AITD patients may be most beneficial in patients with early AITD (TSH ≤ 10 mIU/L). It must, however, be recalled that patients in Group-B (TSH > 10 mIU/L) but not Group-A received levothyroxine supplementation, which may also have some impact on TPO-Ab titers.

Limitations of this study include the open-labeled design of RCT, lack of use of placebo, short follow-up, and assessment of outcomes based on TPO-Ab titers. However, as of today, serum TPO-Ab titers are perhaps the best measures of thyroid autoimmunity in AITD. Reduction in TPO-Ab titers in patients with TSH ≤ 10 mIU/L (who did not receive levothyroxine) underpins the role of Vitamin D in bringing about this reduction. This study highlights the need for further long-term interventions with Vitamin D to evaluate if this reduction in TPO-Ab titers translates into reduced progression of subclinical to overt primary hypothyroidism or in inducing remission of hypothyroidism as evidenced by stoppage/reduction of dose of levothyroxine, which would be a more clinically relevant outcome.

Conclusion

To conclude, Vitamin D deficiency and insufficiency is significantly more common among AITD patients in Eastern India. Vitamin D supplementation in these patients is associated with a beneficial effect on autoimmunity as evidence by a significant reduction in circulating TPO-Ab titers.

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Conflicts of interest

There are no conflicts of interest.

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