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Study of 25-Hydroxy vitamin D Levels in Newly Diagnosed Patients of Autoimmune Thyroid Diseases in a Tertiary Care Hospital in Sub-Himalayan Region of North India-A Cross-Sectional, Case Control Study.

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Abstract
Background: *25-Hydroxyvitamin D [25(OH)D] deficiency has been found with increasing prevalence and with increased risk for predisposition to autoimmune thyroid diseases (AITDs).We aim to assess 25(OH)D deficiency and its association with AITDs in sub-Himalayan region of North India, comparatively a colder and less sunshine area.*
Methods: *A total of 150 subjects were allocated to two case groups, each comprising of 50 hypothyroidism with Hashimoto's thyroiditis (HT)and 50 hyperthyroidism with Graves' diseases (GD) patients and compared to 50 euthyroid controls for assessment of 25(OH) D levels.*
Results: *25(OH)D deficiency was found significantly in higher prevalence in hyperthyroidism (GD) patients as compared to hypothyroidism (HT) patients and controls (12.50±6.05,16.65±13.33 and 27.47±12.94 ng/ml; P<0.001) respectively and more frequently in females in all study groups.25(OH)D levels and thyroid peroxidase autoantibodies(TPOAb) positivity revealed strong inverse correlation in both hypothyroidism (HT) and hyperthyroidism (GD) groups (r= -0.723; P<0.001, r = -0.702 P<0.001) respectively.*
Conclusion: *25(OH)D deficiency was significantly associatedwith AITDs and revealed significantinverse correlation with TPOAb positivity, indicating its predisposition to autoimmunity in AITDs. Therefore, 25(OH)D levels are recommended to be assessed in AITDs for early replacement.*
Keywords: *Autoimmune thyroid diseases, Graves' disease,Hashimoto's thyroiditis, Thyroid peroxidase autoantibodies (TPOAb), Vitamin D deficiency.*

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I. Introduction

25-Hydroxy vitamin D[25(OH)D] deficiency is a global health problem affecting over a billion people worldwide.[1] It has been estimated worldwide, ranging from 20-100% in elderly men,women,children,young and middle aged adults.[2,3] In India,25(OH) D deficiency has increasing prevalence, mainly attributed to skin pigmentation and lack of sunshine.[4,5] Hypovitaminosis D has been found widespreadly from southern to northern India affecting school children and healthy adults.[5,6] Understanding of role of 25(OH)D in human health has been evolving since its discovery in the early 20th century.[7] Most important role of 25(OH)D is to maintain calcium and phosphorus homeostasis to preserve bone health apart from its involvement in muscle development and immune functions. The two important sources from which human body mainly obtains 25(OH)D are exposure of the skin to solar ultraviolet-B radiation(wavelength,290-315nm) which converts steroid cholesterol derivative,7-dehydrocholestrol(provitamin D_3) to previtamin D_3 to form vitamin D_3(cholecalciferol) and, the diet or supplements(vitaminD_3,or ergocalciferol;vitamin D_2 from plant sources).25(OH)D is hydroxylated in the liver by Cytochrome P450(CYP2R1) or 25-hydroxylase enzyme to form 25(OH) vitamin D[25(OH)D_3 or calcidiol], an inactive but major circulating form currently used to evaluate 25(OH)D status by clinical laboratories.25(OH)D_3 is converted to its biological active form 1,25-dihydroxy vitamin D[1,25(OH)2 D_3 or calcitriol] in the kidney by Cytochrome P450(CYP27B1) or 1α-hydroxylase enzyme.[8]

25(OH)D has been demonstrated to have a role in non-skelatal disorders mainly, autoimmune thyroid disease(AITDs).[9,10] Hashimoto's Thyroiditis(HT) and Graves' disease(GD) are well interrelated AITDs. The

hallmark of AITDs is circulating antibodies and reactive T cells against one or another thyroid antigens.[11]The prevalence of thyroid stimulating receptor antibody(TSHR-Ab)and thyroid peroxidase autoantibody(TPOAb) in GD and HT are 80-90%;50-80% and 80-90%;90-100% respectively.[12] 25(OH)D have receptor molecular structure similar to thyroid hormone receptors.[13] Gene polymorphisms of several proteins and enzymes associated with 25(OH)D functions, such as vitamin D receptors(VDR), vitamin D binding proteins(VDB), CYP27B1 and CYP2R1 have been found to be associated with thyroid autoimmunity susceptibility.[14] VDR on immune system cells such as monocytes, macrophages, antigen presenting cells(APCs), dendritic cells(DCs) and lymphocytes explains immune modulator role of 25(OH)D. HT is the most common AITD with strong genetic predisposition presenting as hypothyroidism.Several studies have reported 25(OH)D deficiency strongly associated with HT.[15-17] Low 25(OH)D levels may increase the risk of GD. Patients of GD were found more deficit in 25(OH)D in comparison to controls and patients of HT.[18,19] 25(OH)D demonstrated significantly low levels in TPOAb positive than TPOAb negative patients in hypothyroidism.[20,21] An inverse correlation was found between 25(OH)D and TPO positivity in HT.[9,22] The studies also reported negative correlation between thyroid stimulating hormone(TSH) and 25(OH)D and calcium.[23,24]25(OH)D replacement revealed significant reduction in TPOAb in AITDs.[25,26] This further demonstrates the role of 25(OH)D deficiency in increasing the risk of AITDs and that of its consequent replacementin decreasing the autoimmunity with increasing 25(OH) D levels.

II. Methods

A hospital based, cross-sectional, case control study was conducted over a period of one year from July 2019 to June 2020 in a tertiary care hospital situated in the sub-Himalayan region of North India.A total of 150 subjects were studied.100 patients of newly diagnosed thyroid disease of autoimmune etiology were allocated to two case groups comprising each group of 50 patients of hypothyroidism with Hashimoto's thyroiditis(HT) and 50 patients of hyperthyroidism with Graves' disease(GD) from amongst outdoor patients (OPD) of Medicine and Endocrinologyand compared to age and sex matched 50euthyroid controls from amongst OPD patients. After taking approval fromInstitutional ethics committee, the participants were thoroughly evaluated for thyroid profile,TPOAb,USG thyroid and 25(OH)D levels. Chemiluminescence enzyme immunoassay (CLIA) for TSH,FT4 and TPOAb and enhanced Chemiluminescence enzyme immunoassay (eCLIA) for 25 (OH)D were used for estimation of the levels. Patients with TSH levels >5µIU/ml,TPOAb>5.61IU/ml and/or ultrasonography features of thyroiditis were diagnosed as hypothyroidism(HT) and those with TSH levels <0.1 µIU/ml and elevated FT3 and FT4 with TPOAb>5.61IU/ml and/or ultrasonography features of thyroiditis were diagnosed as hyperthyroidism(GD) and individuals with normal thyroid profile and without positive TPOAb were defined as euthyroid controls .25(OH)D levels <20ng/ml,20-29 ng/ml and >30 ng/ml were defined as deficit,insufficient and sufficient respectively.

Inclusion criteria

1. Adults, aged 18 years and above of both sexes in both case and control groups,
2. Incase groups, newly diagnosed patients of autoimmune thyroid disease i.ehypothyroidism (HT) and hyperthyroidism (GD) from amongst OPD patients and
3. In control group ,euthyroid individuals from amongst OPD patients were included in the study for estimation of 25(OH)D levels.

Exclusion criteria

1. Patients, aged less than 18 years,non-consenting and seriously ill,
2. Patients with chronic kidney disease (CKD), chronic liver disease, metabolic bone disorders, primaryhyperparathyroidism, pregnancy, post-partum and other autoimmune diseases and
3. Patients on 25(OH)D replacement,glucocorticoids, antiepileptics, antitubercular, anti-osteoporotic, antipsychotics, amiodarone and iatrogenic (post radiation) therapy in both cases and control groups were excluded from the study.

Statistical Analysis

Data of both cases and control groups were collectedand entered into Microsoft excel spreadsheet and transferred to Epi info7.1 software for analysis.Continuous variables were expressed as mean and standard deviations while categorical variables as proportions, percentages and 95% confidence interval (CI). Chi squaretest($\chi 2$) for comparison of categorical variables and students't' test for comparison of means were used. For association p value<0.05 was considered as statistically significant.

III. Results

A total of 150 subjects were studied over a period of one year from July 2019 to June 2020 comprising newly diagnosed patients of AITDs allocated to two case groups, each of 50 patients of hypothyroidism(HT) and 50 patients of hyperthyroidism(GD) and 50 euthyroid controls .The age of patients ranged from 19 to 80 years with mean age of 42.28±14.67, 39.26±8.90 and 39.24 ± 11.90 years and male vs female ratio(36% vs 64%, 36% vs 64% and 42% vs 58%) in hypothyroidism(HT), hyperthyroidism(GD) and in control groupsrespectively(Table 1).

Table 1: Age and sex distribution of case and control groups.

Parameters		Hypothyroidism(HT) No=50 (33.3%)	Hyperthyroidism(GD) No =50 (33.3%)	Control No=50 (33.3%)
Age(years) Mean±SD		42.28 ± 14.67	39.26 ±8.90	39.24 ± 11.90
Sex	Male	18 (36.0%)	18 (36.0%)	21 (42.0%)
	Female	32 (64.0%)	32 (64.0 %)	29 (58.0%)

HT, Hashimoto's thyroiditis; GD, Graves' disease.

Each group ofhypothyroidism (HT), hyperthyroidism(GD) and controls revealed TSH levels of 26.54±25.69,0.02±0.02 and 2.80±1.17 µIU/ml and TPOAb levels of 635.76±366.27,566.92±239.63 and 0.00±0.00IU/ml, respectively(P<0.001;Table 2). Ultrasonographic features of thyroiditis were found in 33(66%) patients of hypothyroidism (HT) and 2(4%) patients of hyperthyroidism (GD) and none in control group.

Table 2: Comparison of TSH and TPOAb among case and control groups.

Groups	TSH (µIU/ml) Mean± SD	TPOAb (IU/ml) Mean ± SD	USG Thyroid	
			Enlargement N (%)	Thyroiditis N (%)
Hypothyroidism(HT) No=50	26.54 ±25.69	635.76 ± 366.27	22(44.0%)	33(66.0%)
Hyperthyroidism(GD) No=50	0.02 ±0.02	566.92 ± 239.63	23(46.0%)	2(4.0%)
Control No=50	2.80 ± 1.17	0.00 ± 0.00	0(0.0%)	0(0.0%)
P value	<0.001*	0.269	<0.001*	<0.001*

*Significant;HT, Hashimoto's thyroiditis; GD, Graves' disease; TSH, Thyroidstimulatinghormone ;TPOAb , Thyroid peroxidase autoantibodies.

25(OH)D revealed levels of 16.65±13.33, 12.50±6.05 and 27.47±12.94 ng/ml in hypothyroidism(HT), hyperthyroidism(GD) and control groups respectively(Table3).25(OH)D levels were significantly decreased in patients of hypothyroidism(HT) and hyperthyroidism(GD) as compared to control group(P<0.001).

Table 3: Comparison of 25(OH)D levels among case and control groups.

Groups	25 (OH) D (ng/ml) Mean±SD	Mean difference	P value
Hypothyroidism(HT) N=50	16.65±13.33	4.15	0.048*
Hyperthyroidism(GD) N=50	12.50±6.05		
Hypothyroidism(HT) N=50	16.65±13.33	10.82	<0.001*
Control N=50	27.47±12.94		
Hyperthyroidism(HT) N=50	12.50±6.05	14.97	<0.001*
Control N=50	27.47±12.94		

*Signifincant;HT, Hashimoto's thyroiditis; GD, Graves' disease; 25(OH)D, 25-Hydroxy vitamin D.

Deficit25(OH)D(<20 ng/ml)were found in 36(72%) with female vs male;22(44%) vs 14(28%) in hypothyroidism(HT) patients,in 42(84%) with female vs male; 26(52%) vs 16(32%) in hyperthyroidism(GD) patients and in 15(30%) and with female vs male; 10(20%) vs 5 (10%) in control group(Table4 and Figure 1).

Table 4 : Comparison of 25(OH) D deficiency , insufficiency and sufficiency among case and control groups.

Group	Gender	Deficiency 25(OH)D (<20ng/ml) N(%)	Insufficiency 25(OH)D (20-29 ng/ml) N (%)	Sufficiency 25(OH)D (>30 ng/ml) N (%)
Hypothyroidism(HT) N=50	Male	14 (28.0%)	3(6.0%)	1(2.0%)
	Female	22(44.0%)	6(12.0%)	4(8.0%)
	Total	36(72.0%)	9(18.0%)	5(10.0%)
Hyperthyroidism(GD) N=50	Male	16(32.0%)	2(4.0%)	0(0.0%)
	Female	26(52.0%)	6(12.0%)	0(0.0%)
	Total	42(84.0%)	8(16.0%)	0(0.0%)
Control N=50	Male	5(10.0%)	4(8.0%)	12(24.0%)
	Female	10(20.0%)	10(20.0%)	9(18.0%)
	Total	15(30.0%)	14(28.0%)	21(42.0%)

HT, Hashimoto's thyroiditis; GD, Graves' disease; 25(OH)D,25-Hydroxy vitamin D.

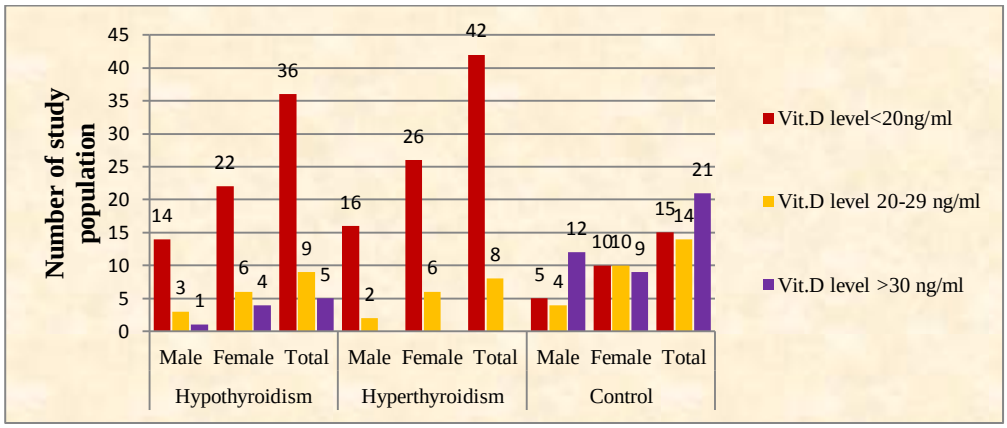

Figure1 : Comparison of 25(OH) vitamin D deficiency , insufficiency and sufficiency among case and control groups.Vit D, 25-Hydroxy vitamin D.

25(OH)D deficiency was found more frequently in hyperthyroidism (GD) patients followed by hypothyroidism (HT) patients as compared to control groupand also more frequently in females in all the three study groups. 25(OH)D deficiency wasfound more frequently than its insufficiency (20-29 ng/ml) in all the three study groups. All the patients revealed 25(OH)D deficiency and insufficiency in hyperthyroidism (GD) group suggesting more pronounced autoimmunity (Table 4 and Figure 1).

Table 5: Pearson's correlation and Linear regression analysis between Anti-TPOAb and 25(OH)D levels in Hypothyroidism (HT)and Hyperthyroidism (GD).

Groups	Regression Equations	Correlation(r)	P value	R^2 value	SE
Hypothyroidism (HT)	25(OH)D level = 33.39 - 0.02633 Anti-TPOAb	-0.723	0.001	52.32%	9.302
Hyperthyroidism(GD)	25(OH)D level = 22.55 - 0.01772 Anti-TPOAb	-0.702	0.001	49.35%	4.348

HT, Hashimoto's thyroiditis; GD, Graves' disease; 25(OH)D,25-Hydroxy vitamin D; TPOAb, Thyroid peroxidase autoantibodies.

25(OH)D and TPOAb levels were correlated using Pearson's correlation and linear regression analysis in both hypothyroidism(HT) and hyperthyroidism(GD) which revealed strong inverse correlation(r=-0.723;P<0.001,r=-0.702;P<0.001) respectively(Table 5 and Figure 2 and 3).

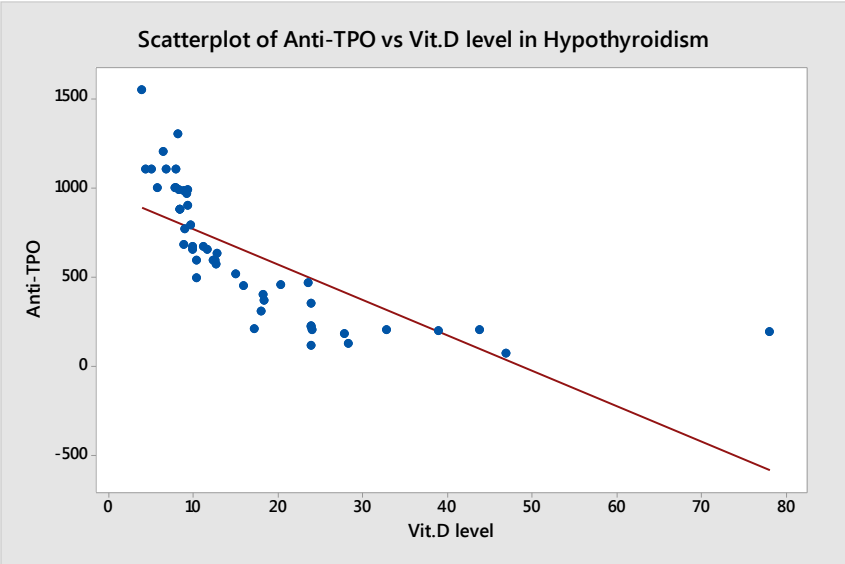

Figure2: Scatterplot of anti-TPO vs vitamin D levels in hypothyroidism (HT). Anti TPO, Thyroid peroxidase autoantibodies; Vit.D, 25-Hydroxy vitamin D.

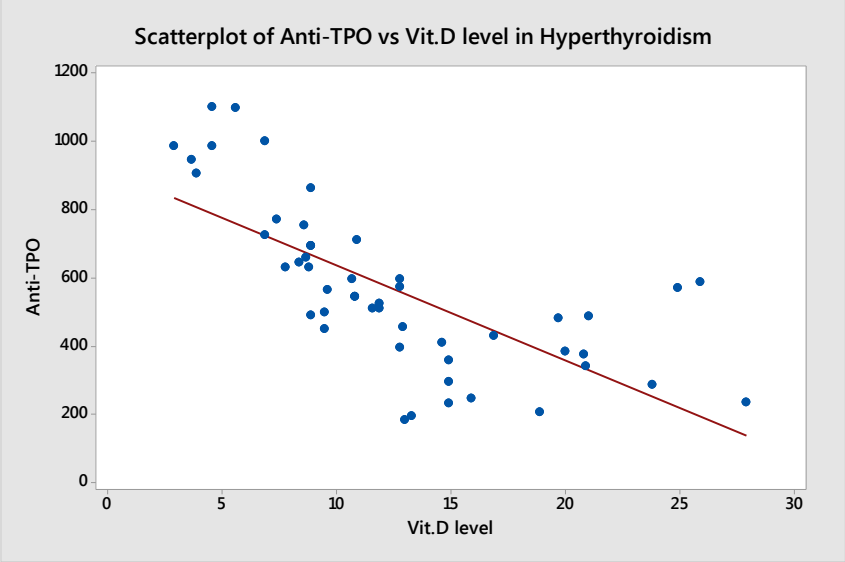

Figure3 : Scatterplot of anti-TPO vs vitamin D levels in hyperthyroidism(GD). Anti TPO, Thyroid peroxidase autoantibodies; Vit.D, 25-Hydroxy vitamin D.

25(OH)D deficiency was found in all the three study groups, more frequently in females and significantly associated with AITDs.

III. Discussion

Thyroid diseases are among the most common endocrine abnormalities and AITDs are the most prevalent autoimmune diseases.[27,28]The present, cross-sectional, case control study was conducted in a tertiary care hospital situated in sub-Himalayan region of northern India, relatively a colder and less sunshine region and recruited newly diagnosed patients of AITDs and euthyroid controls. In this study, a total of 150 subjects had age ranged from 19-80 years with mean age of 42.28± 14.67 years in 50 hypothyroidism(HT),39.26±8.90 years in 50 hyperthyroidism(GD) patients of two case groups and 39.24±11.90 years in control group with male to female ratio of 36% vs 64% in each case group and 42% vs 58% in control group (Table 1).The case and control randomization and demographic characteristics of the study closely matched to a study conducted in 2014, by Unal et al,[19] which recruited newly diagnosed Hashimotos' thyroiditis(HT) and Graves' disease(GD) patients

and compared to healthy controls. Age and sex distribution of this study also closely matched with that of a study conducted by Goswami et al,[9] in India(age range16-60 years, male vs female ; 38% vs 61%).In the present study ,TPOAb was present in HT and GD case groups(635.76± 366.27 and 566.92±239.63 IU/ml) respectively indicating autoimmunity and negative in control group (Table 2).

25(OH)D in the present study revealed significantly lower levels in hyperthyroidism(GD) group(12.50±6.05 ng/ml) as compared to hypothyroidism(HT) group (16.65±13.33 ng/ml; P=0.048) and control group (27.47±12.94 mg/ml; P<0.001) and also significantly lower in hypothyroidism(HT) group than controls(P<0.001;Table 3).25(OH)D levels in the study revealed significantly lower levels in AITDs. The study by Unal et al,[19] revealed 25(OH)D levels of 14.9±8.6 and 19.4±10.1 ng/ml in HT and GD group as compared to controls(22.5±15 ng/ml), significantly lower in case groups.(P<0.001).In thepresent study, 25(OH)D deficiency was found more frequently in hyperthyroidism(GD) patients as compared to hypothyroidism(HT) and control groups(84%,72% and 30%) respectively and also more frequently in females with female vs male (52% vs 32%, 44% vs 28% and 20% vs 10%) respectively and similarly in hypothyroidism (HT) patients and also in female vs male as compared to controls. Similarly hyperthyroidism(GD) group revealed more frequency of 25(OH) D deficiency than insufficiency(84% vs 16%) as compared to hypothyroidism(HT) and control groups (72% vs 18% and 30% vs 28%) respectivelyindicative of more autoimmunity and lower 25(OH) D in GD (Table 4 figure 1).Studies also revealed higher prevalence of lower 25(OH) D levels in patients of GD in comparison to controls.[18,19,29] A significant association could be demonstrated between 25(OH) D insufficiency in autoimmune hypothyroidism in both younger and females of reproductive age group but not in males and postmenopausal females.[30] A study from south India revealed 25(OH)D deficiency more frequently in females vs males (70% vs 44%) in rural and (75% vs 62%) in urban.[5]

Pearson's corelation and linear regression analysis between 25(OH) D and TPOAb positivity revealed significant inverse correlation in both hypothyroidism(HT) and hyperthyroidism(GD) groups(r=-0.723;P<0.001, r=-0.702;p<0.001) respectively(Table 5 and figures 2 and 3).Similar corelation between 25(OH) D and TPOAb positivity was found in studies.[9,19] However, some studies revealed weaker corelation between 25(OH)D and TPOAb positivity.[26,30]Our study in northern region, revealed significant association of 25(OH)D deficiency with AITDs, more frequently in females as compared to a study from north India which observed weaker association of 25(OH)D with TPOAb and nonsignificant difference of its levels in male vs female (85.5% vs 88.2%;P= 0.169).[9]

25(OH)D replacement in the dosesof 60,000 IU weekly and calcium in the dose of 500 mg daily for 3 months has been found to reduce TPOAb significantly in case vs controls (-46.73% and -16.6%).[25]Similarly, 25 (OH) D replacement in daily doses of 1000 IU has also been demonstrated to reduce TPOAb significantly.[26] The role of 25(OH)D replacement in reducing TPOAb has further demonstrated the strong association of vitamin D deficiency and autoimmunity in AITDs.Our study expectedly revealed comparatively higher prevalence of 25(OH)D deficiency in AITDs in relatively, a colder, hilly and less sunshine region of north India.

IV. Conclusion

In the present study, 25(OH)D deficiency has been found significantly associated with AITDSwhich revealed significant inverse correlation with TPOAb positivityin both hypothyroidism (HT) and hyperthyroidism (GD) patients indicating its predisposition to autoimmunity in AITDs. Therefore, 25(OH)D levels are recommended to be assessed in all AITDs patients for early replacement. However, a larger case control study needs to be conducted to evaluate the magnitude of 25(OH)D deficiency prevalence in AITDs and role of itsreplacement as a preventive and therapeutic measure.

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References

[1]. Hollick MF, Chen TC. Vitamin D deficiency a worldwide problem with health consequences. Am J ClinNutr 2008; 87:1080S–1086S.
[2]. Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G, et al. The prevalence of vitamin D deficiency amongst women with osteoporosis: an international epidemiological investigation. J Intern Med 2006; 260: 245–54.
[3]. Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. Arch PediatrAdolesc Med 2004; 158: 531– 7.
[4]. Goswami R, Mishra SK, Kochuhupillai N. Prevalence and potential significance of vitamin D deficiency in Asian Indians. Indian J Med Res 2008;127: 229-38.
[5]. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D, Srinivasarao P V, Sarma K V, et al. High prevalence of low dietary calcium, highphytate consumption, and vitamin D deficiency in healthy south Indians. Am J ClinNutr 2007;85: 1062-7.
[6]. Marwaha RK, Tandon N, Reddy DR, Aggarwal R, Singh R, Sawhney R C, et al. (Vitamin D and bone mineral density status of healthy school children in northern India, Am J ClinNutr 2005;82: 477-82.
[7]. Deluca HF. Evolution of our understanding of vitamin D. Nutr Rev 2008; 66(10):73–87.

[8]. Holick MF, Garabedian M 2006 Vitamin D photobiology, metabolism, mechanism of action, and clinical applications. In; Favus MJ, ed. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism. 6th ed. American Society for Bone and Mineral Research, Washington, DC; 2006: pp129-37.
[9]. Goswami R, Marwaha RK, Gupta N, Tandon N, Sreenivas V, Tomar N, et al . Prevalence of vitamin D deficiency and its relationship with thyroid autoimmunity in Asian Indians: a community-based survey. Br J Nutr 2009;102:382–6.
[10]. Muscogiuri G, Mari D, Prolo S, Fatti LM, Cantone MC, Garagnani P, et al . 25 hydroxyvitamin D deficiency and its relationship to autoimmune thyroid disease in the elderly. Int J Environ Res Public Health 2016; 13(9): 850-6.
[11]. Weetman AP. Autoimmune thyroid disease. Autoimmunity 2004 ;37(4):337-40.
[12]. Domenico S, Ronald C, Peter A, P Reedlarsen. Thyroid pathophysiology and diagnostic evaluation. In: Sholmo M, Richard J, Allison B, Clliford R, eds. Williams Textbook of Endocrinology. 14th ed. Philadelphia, PA: Elsevier; 2015: pp 361-2.
[13]. McDonnell DP, Pike JW, O'Malley BW. The vitamin D receptor: a primitive steroid receptor related to thyroid hormone receptor. J Steroid Biochem 1988; 30: 41–6 .
[14]. Mazokopakis EE, Kotsiris DA. Hashimoto's autoimmune thyroiditis and vitamin D deficiency.Current aspects. Hell J Nucl Med 2014; 17:37-40.
[15]. Tamer G, Arik S, Tamer I, Coksert D. Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid 2011; 21(8):891-6.
[16]. BozkurtNC,KarbekB,UcanB,SahinM,CakalE,Ozbek M, et al. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis.EndocrPract 2013; 19: 479–84.
[17]. Shin DY, Kim KJ, Kim DH, Wang S, Lee EJ. Low serum vitamin D is associated with anti-thyroid peroxidase antibody in autoimmune thyroiditis. Yonsei Med J 2014 ;55(2):476-81.
[18]. Xu MY, Cao B, Yin J, Wang DF, Chen KL, Lu QB. Vitamin D and Graves' disease: A meta-analysis update.Nutrients 2015; 7: 3813–27.
[19]. Unal AD, Tarcin O, Parildar H, Cigerli O, Eroglu H, Demirag NG. Vitamin D deficiency is related to thyroid antibodies in autoimmune thyroiditis. Cent Eur J Immunol 2014; 39(4):493–7.
[20]. Khare J, Reddy B, Nalla S, Srivastava P, Wadhwa J, Deb P. Vitamin D deficiency and its association with thyroid peroxidase antibodies positive hypothyroidism-Experience in a tertiary center in South India. Med J Dr. DY Patil University 2017;10(4):355-8.
[21]. De Pergola G, Triggiani V, Bartolomeo N, Giagulli VA, Anelli M, Masiello M, et al . Low 25 hydroxyvitamin D levels are independently associated with autoimmune thyroiditis in a cohort of apparently healthy overweight and obese subjects.EndocrMetab Immune Disord Drug Targets 2018; 18:646–52.
[22]. Mansournia N, Mansournia MA, Saeedi S, Dehghan J. The association between serum 25OH D levels and hypothyroid Hashimoto's thyroiditis.JEndocrinolInvestig 2014; 37 (5):473-6.
[23]. Sinha R, Bhushan I. The study of serum calcium and 25-OH vitamin D levels in newly diagnosed hypothyroid patients. . IOSR J Dent Med Sci 2019; 18:18–21.
[24]. Singh D, Alam MR. Evaluation of deficiency of vitamin D and low level of serum calcium in hypothyroid patients. Int J Health Clin Res 2020 ; 3(12):184-7.
[25]. Chaudhary S, Dutta D, Kumar M, Saha S, Mondal SA, Kumar A, et al. Vitamin D supplementation reduces thyroid peroxidase antibody levels in patients with autoimmune thyroid disease: An open-labeled randomized controlled trial. Indian J EndocrinolMetab 2016; 20: 391–8.
[26]. Simsek Y, Cakır I, Yetmis M, Dizdar OS, BaspinarO,GokayF.Effects of vitamin D treatment on thyroid autoimmunity. J Res Med Sci 2016; 21: 85-90.
[27]. Shapira Y, Agmon-Levin N, Shoenfeld Y. Geoepidemiology of autoimmune rheumatic diseases. Nat Rev Rheumatol 2010; 6:468–76.
[28]. Shoenfeld Y, Selmi C, Zimlichman E, Gershwin ME . The autoimmunologist: geoepidemiology, a new center of gravity, and prime time for autoimmunity. J Autoimmun 2008; 31: 325–30.
[29]. Wang J, Lv S, Chen G, Gao C, He J, Zhong H, et al. Meta-analysis of the association between vitamin D and autoimmune thyroid disease. Nutrients 2015 ;7(4):2485-98.
[30]. Christopher S, Santhanavally RN. Study on association between vitamin D deficiency and autoimmune hypothyroidism.J Med SciClin Res2019;7:258-65.

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