Evaluating correlation between Vitamin D levels and hypothyroidism: A pilot study

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Abstract
Vitamin D deficiency is very common worldwide. Vitamin D has steroid like properties being produced in skin and regulates the expression of various genes. Its main biochemical role is in the regulation of bone metabolism by regulation of calcium and phosphorus levels in the body. Vitamin D has non-skeletal functions also like immunomodulation and anti-inflammation which could be the reason behind the pathogenesis of thyroid diseases. Several studies have proved the role of vitamin D in the pathogenesis of various diseases like autoimmune diseases, infectious diseases, heart diseases, metabolic syndrome as well as cancer. Considering this potential role, this study was done with the aim of assessing Vitamin D levels and correlation of these levels of vitamin D with thyroid profile in patients of hypothyroidism.

Keywords: Vitamin D, Hypothyroidism, Thyroid profile, Autoimmune thyroid disease, Vit D deficiency.

Introduction
Hypothyroidism is one of the most common endocrine disorders. In past, the most primary cause of hypothyroidism was iodine deficiency; however, with the introduction of iodized salt, its incidence was reduced in the industrialized nations. The prevalence of hypothyroidism in developed countries is about 4%-5%, whereas in India, it is reported to be around 10.95%. As per the epidemiology study conducted by Unnikrishnan et al, in eight cities of India, the prevalence of subclinical hypothyroidism, a mild thyroid failure was found to be 8.02%. The prevalence of subclinical hypothyroidism ranges between 4%-15% worldwide and is reported to be 11.4% for women and 6.2% for men in India.[1]

In areas where iodine sources are sufficient, autoimmune hypothyroidism is more common than iodine deficient thyroid diseases. Hashimoto's thyroiditis is a hereditary disorder mostly seen in middle aged US population. Most of the Hashimoto's thyroiditis patients (90-100%) have presence or high levels of anti thyroid antibodies. In India autoimmune hypothyroidism is the most frequent endocrine disorder affecting more than 42 million people.[2] Intrathyroidal lymphocytic infiltration is followed by a gradual destruction of the thyroid gland which may lead to subclinical or overt hypothyroidism. Biochemical markers of the disease are thyroid peroxidase and/or thyroglobulin autoantibodies in the serum. Vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in Vitamin D receptor was shown to predispose people to autoimmune thyroid diseases including Grave's disease and Hashimoto's thyroiditis.[3]

Previously, it has been considered that vitamin D deficiency was rare in India because of enough exposure to sunshine. A study carried out in Delhi on study population included newborns and their mothers, health care workers, soldiers and patients of albinism and vitiligo presented with 25-hydroxy vitamin D (25(OH) D) insufficiency or deficiency.[4] Based on these study groups, subnormal serum 25(OH) D levels of Asian Indians could be linked to their skin pigmentation and poor sunshine exposure.

Studies assessing the association between autoimmune thyroid disease & vitamin D levels have produced conflicting results.[5-8] In India, few studies had done to explain the relationship between vitamin D levels & thyroid autoimmunity. Considering the high prevalence of thyroid autoimmunity and vitamin D deficiency in the Indian population it arises a need to assess the relationship between these two diseases. So, the present study was conducted with the aim to estimate the levels of vitamin D in patients with hypothyroidism and to correlate the levels of vitamin D with thyroid profile in these patients

Materials and Methods
This cross sectional observational pilot study was conducted in the Department of Biochemistry in collaboration with the Department of Medicine, in a Tertiary Care Hospital. After taking prior ethical clearance by the institutional ethical committee, the study was conducted from 1st June, 2017 to 31st July, 2017.

Hypothyroid patients in the age group of 21-50 years of either sex that visited Medicine OPD during this period were included in the study after their informed consent. The patients with clinical history and laboratory investigations suggestive of hypothyroidism with TSH values > 4.68 mIU/ml were included in the study. The patients with post radio–iodine hypothyroidism, liver disorders, renal disorders or primary hyperparathyroidism, those on anti-epileptic medication or vitamin D supplementation were excluded from the study.

Sample Collection
After 12 hours of fasting, venous blood sample was collected under aseptic conditions and processed within 24 hours for the following parameters:

Hemoglobin (Hb): Measured by cyanmethemoglobin method.
Erythrocyte sedimentation rate (ESR): Measured by automated micro ESR system.
Random Blood Glucose (RBS): Measured by hexokinase method on Siemen's RxL Dimensions.
Serum Calcium: Measured by calcium o-cresolphthalein complexone (OCPC) method on Siemen's RxL Dimensions.
Serum Phosphorous: Measured by modification of classical phosphomolybdate method on Siemen's RxL Dimensions.
Alkaline Phosphatase: Measured by p-nitrophenol method on Siemen's RxL Dimensions.
Free T3: Measured by enhanced chemiluminescence technique on Integrated Vitros 56oo. The reference range is 2.32 – 6.16 pg/mL.
Free T4: Measured by enhanced chemiluminescence technique on Integrated Vitros 56oo. The reference range of 0.78-2.19 ng/dL.
TSH: Measured by enhanced chemiluminescence technique on Integrated Vitros 56oo. The reference range is 0.46-4.68 mIU/L.
Vitamin D: Measured by competitive enhanced chemiluminescence assay technique on Integrated Vitros 5600. The reference range is <20 ng/mL (deficient), 20-29 ng/mL (insufficient), 30-100 ng/mL (sufficient) and >100 ng/mL (potential toxicity).

Statistical Analysis
The data thus obtained was analyzed statistically using SPSS software version 16 for windows. Results of various parameters were presented as mean ± standard deviation (SD). The statistical correlation in vitamin D levels and other biochemical parameters including thyroid profile was tested using pearson's correlation analysis.

Results
It was observed that all the newly diagnosed patients of hypothyroidism visiting the OPD during the duration of study were females (24 patients). The descriptive statistics (mean ± SD) of blood investigations is shown in Table 1. Pearson's correlation analysis between different blood investigations is given in Table 2. The levels of vitamin D were insufficient in patients with hypothyroidism with mean ± SD of 22.01± 10.83 as shown in Table 3. Pearson's correlation analysis between vitamin D level and thyroid profile shows that vitamin D has a negative correlation with TSH, free T3 and free T4 level which signifies that with the elevation in the serum TSH levels, serum 25(OH)D level falls in these patients. (Table 4).

Table 1: Descriptive statistics of blood investigations

S. No.	Parameter	Mean ± SD
		N=24
1	Hb (g/dL)	11.27 ± 1.42
2	ESR (mm/hr)	24.59 ± 12.29
3	RBS (mg/dL)	111.91 ± 33.50
4	Cal (mg/dL)	9.37 ± 0.73
5	Phos (mg/dL)	3.85 ± 0.86
6	ALP (U/L)	87.77 ± 23.85

Table 2: Correlation between different blood parameters

		Hb	ESR	RBS	TSH	FT3	FT4	Vit D	Cal	Phos	ALP
Hb	**Pearson Correlation**	1	-0.213	0.206	0.197	-0.338	-0.337	0.000	0.062	-0.039	0.282
	Sig. (2-tailed)		0.341	0.358	0.381	0.124	0.125	0.999	0.785	0.864	0.204
ESR	**Pearson Correlation**	-0.213	1	0.062	-0.143	0.129	0.033	-0.425*	0.254	-0.140	0.239
	Sig. (2-tailed)	0.341		0.784	0.527	0.568	0.886	0.049	0.254	0.534	0.284
RBS	**Pearson Correlation**	0.206	0.062	1	-0.040	0.020	-0.095	0.123	0.118	-0.497*	0.008
	Sig. (2-tailed)	0.358	0.784		0.859	0.929	0.674	0.584	0.600	0.019	0.973
TSH	**Pearson Correlation**	0.197	-0.143	-0.040	1	-0.401	-0.354	-0.215	-0.090	-0.041	0.226
	Sig. (2-tailed)	0.381	0.527	0.859		0.065	0.106	0.337	0.689	0.857	0.313
FT3	**Pearson Correlation**	-0.338	0.129	0.020	-0.401	1	0.740**	-0.055	0.249	0.293	0.146
	Sig. (2-tailed)	0.124	0.568	0.929	0.065		0.000	0.809	0.263	0.186	0.517
FT4	**Pearson Correlation**	-0.337	0.033	-0.095	-0.354	0.740**	1	-0.090	-0.014	0.355	-0.042
	Sig. (2-tailed)	0.125	0.886	0.674	0.106	0.000		0.691	0.952	0.105	0.853
Vit D	**Pearson Correlation**	0.000	-0.425*	0.123	-0.215	-0.055	-0.090	1	0.130	-0.182	-0.493*
	Sig. (2-tailed)	0.999	0.049	0.584	0.337	0.809	0.691		0.565	0.417	0.020
Cal	**Pearson Correlation**	0.062	0.254	0.118	-0.090	0.249	-0.014	0.130	1	0.030	0.107
	Sig. (2-tailed)	0.785	0.254	0.600	0.689	0.263	0.952	0.565		0.895	0.635
Phos	**Pearson Correlation**	-0.039	-0.140	-0.497*	-0.041	0.293	0.355	-0.182	0.030	1	0.251
	Sig. (2-tailed)	0.864	0.534	0.019	0.857	0.186	0.105	0.417	0.895		0.260
ALP	**Pearson Correlation**	0.282	0.239	0.008	0.226	0.146	-0.042	-0.493*	0.107	0.251	1
	Sig. (2-tailed)	0.204	0.284	0.973	0.313	0.517	0.853	0.020	0.635	0.260	

*.Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

Table 3: Descriptive statistics of thyroid profile and vitamin D

S. No.	Parameter	Mean ± SD
		N=24
1	TSH (mIU/L)	16.35 ± 25.14
2	FT3 (pg/mL)	3.08 ± 0.61
3	FT4 (ng/dL)	0.89 ± 0.18
4	Vitamin D (ng/mL)	22.01 ± 10.83

Table 4: Correlation between thyroid profile and vitamin D

		TSH	FT3	FT4	Vit D
TSH	**Pearson Correlation**	1	-0.401	-0.354	-0.215
	Sig. (2-tailed)		0.065	0.106	0.337
FT3	**Pearson Correlation**	-0.401	1	0.740**	-0.055
	Sig. (2-tailed)	0.065		0.000	0.809
FT4	**Pearson Correlation**	-0.354	0.740**	1	-0.090
	Sig. (2-tailed)	0.106	0.000		0.691
Vit D	**Pearson Correlation**	-0.215	-0.055	-0.090	1
	Sig. (2-tailed)	0.337	0.809	0.691	

**. Correlation is significant at the 0.01 level (2-tailed).

Discussion

The current study involved the analysis of different blood parameters, especially vitamin D levels, in patients with newly diagnosed hypothyroidism. In the present study, it was observed that hypothyroidism is more prevalent in females than males. Similar observations were also observed in the studies conducted by Wu P et al and Wiersinga WM et al.[9,10] It was observed that the haemoglobin levels were below normal in the study population. The studies conducted by Wang et al[11] and Dorgalaleh et al[12] showed similar results, the patients with hypothyroid had Hb levels less than 12 g/dL in both males as well as females. The ESR level of the patients was raised. This observation was in accordance with Savas et al study.[13] The hypothyroid patients had a higher ESR levels. The level of alkaline phosphatase, phosphorous and calcium were in the normal range in the study group.

The level of vitamin D in hypothyroid patients was low with a mean of 22.01 ng/mL. The results were supported by the study of Mackawy et al., Kivity et al., and Effraimidis et al.[3,5,14] The free T3 and T4 level was negatively correlated with TSH and thus an increase in TSH level was followed by a decrease in free T3 and T4 level. The vitamin D level was negatively correlated with TSH level. The reason for this finding could be that both vitamin D and thyroid hormones bind with the similar receptors called steroid receptors. Vitamin D mediates its action by binding to vitamin D receptors (VDR) and then activation of VDR-responsive genes. So, VDR gene polymorphism can lead to autoimmune thyroid disease and it is one of the culprits for the onset of a number of autoimmune diseases including hypothyroidism.[3]

Autoimmune hypothyroidism is characterized by lymphocytic infiltration of thyroid gland and synthesis of pathogenic thyroid autoantibodies. Pathogenesis of autoimmune thyroid diseases is multifactorial. The mechanism for the association of vitamin D deficiency in hypothyroidism is probably because of immunoregulatory and anti-inflammatory property of vitamin D. The immunomodulatory property of vitamin D is because of its effect on innate and adaptive response. Vitamin D inhibits the production of Th1 cytokine (IL-12), therefore shift the polarization of T cells from Th1 to Th2. In CD4+ T cells, Vitamin D directly inhibits the production of TH1 cytokines (IL-2 and IFN-γ) and promotes the production of Th2 cytokine (IL-4 and IL-5).[15]

A negative correlation between vitamin D and free T3 and T4 levels was also observed. This maybe because the level of free T3 and T4 were within the normal range in newly diagnosed cases. The study therefore suggests a correlation between vitamin D deficiency and hypothyroidism.

Limitations

1. The study was done in a period of 2 months only.
2. Possibility of selection bias because only those patients were enrolled who visited tertiary hospital.

Conclusion

This study concluded that the vitamin D insufficiency was observed in hypothyroid patients. A negative correlation between serum TSH and vitamin D level was observed. The finding suggests that vitamin D should be thoroughly kept in normal range in order to control the levels of thyroid hormones. Future studies would include the analysis of genetic link between vitamin D and hypothyroidism.

Conflict of Interest: None.

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