



**RESEARCH ARTICLE**

**VITAMIN D LEVELS CORRELATED WITH HYPOTHYROIDISM IN INDIAN POPULATION: A PILOT STUDY**

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**ABSTRACT**

**Background:** Vitamin D is a pro-hormone that not only regulates blood calcium levels, but has many other beneficial actions.

**Objective:** We aim to estimate serum 25(OH) Vitamin D and i-PTH (intact-parathyroid hormone) levels in hypothyroidism.

**Methods:** 100 subjects were included in the study and patients having serum hTSH > 10 µIU/L or serum hTSH levels between 6 to 10 µIU/L along with decreased serum FT3 &/or FT4 levels were diagnosed as hypothyroid cases. Serum 25(OH) Vitamin D was estimated using ELISA. Serum hTSH & serum i-PTH assay was based on chemiluminescence. Statistical analysis was done (SPSS).

**Results:** We observed that 25(OH) Vitamin D levels were significantly lower in hypothyroid patients ( $20.0 \pm 1.49$  ng/ml) compared to controls ( $27.7 \pm 1.34$  ng/ml),  $p < 0.001$ . A negative and significant correlation was observed between 25(OH) Vitamin D and hTSH levels ( $r = -0.48$ ,  $p < 0.001$ ). i-PTH levels were significantly increased in hypothyroid patients ( $57.6 \pm 3.30$  pg/ml) vs ( $36.8 \pm 1.91$  pg/ml), though a significant correlation was not observed between i-PTH & hTSH among cases ( $r = 0.11$ ,  $p = 0.43$ ).

**Conclusions:** Hypothyroid patients frequently bespeak a Vitamin D deficient state which suggests that they need routine screening and supplementation for the same.

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**INTRODUCTION**

Over the last few years, the sunshine vitamin or Vitamin D has gained a lot of attention. Though initially thought to be uncommon, the reported prevalence of Vitamin D insufficiency in India is around 50-90 % (Harinarayan and Joshi, 2009). Despite enough sunshine, this unexpected insufficiency of Vitamin D levels among Indians has become a matter of concern (Londhey, 2011). Besides the well known role in skeletal system, its role has recently been implicated in cardiovascular system, cancer and several autoimmune disorders including Diabetes mellitus (Giovannucci *et al.*, 2008; Zella and Deluca, 2013; Komorowski *et al.*, 2013). According to a projection, it has been estimated that about 42 million people in India suffer from thyroid diseases (Usha *et al.*, 2009). Prevalence of hypothyroidism has risen markedly in the last few decades affecting even younger age groups in the form of congenital hypothyroidism (Unnikrishnan and Usha, 2011).

The main active form of Vitamin D ; 1,25(OH)<sub>2</sub> Vitamin D is synthesized following hydroxylations in liver and kidney which is then taken up by several target cells that possess Vitamin D receptor (VDR). However, it is the less-active 25(OH) Vitamin D, precursor form of active Vitamin D that provides an estimate for the body reserve as it has a longer half-life. Both normal and decreased concentrations of 25(OH) Vitamin D have been found in thyroid patients (Bouillon *et al.*, 1980; Mosekilde *et al.*, 1977). Vitamin D deficiency is

considered to be present when serum 25(OH) Vitamin D levels are < 20ng/ml, insufficiency between 20-30ng/ml and sufficient when levels are above 30ng/ml (Holick, 2007). Initially thought to be vitamin just regulating calcium homeostasis, research has shown that this steroid hormone affects more than 36 cell types which possess VDR including thyroid gland (Feldman *et al.*, 2005).

Vitamin D exerts its metabolic effects on skeletal, cardiovascular and reproductive systems. So, a lower level of Vitamin D is likely to aggravate the systemic abnormalities associated with hypothyroidism (Wang *et al.*, 2008; Chopra *et al.*, 2011). The intact-PTH (i-PTH) levels rise in subjects with insufficient levels of Vitamin D body reserves to maintain serum calcium balance. Both Vitamin D and thyroid hormone act through steroid receptors and may affect each other's action as they have similar response elements on genes. It is still unclear if any association exists between hypothyroidism and Vitamin D insufficiency. We hypothesize that hypothyroid subjects are more likely to develop Vitamin D deficiency and require routine screening and supplementation.

The aim of the present study was to estimate serum 25(OH) Vitamin D and serum i-PTH (intact-parathyroid hormone) concentrations in newly diagnosed hypothyroid patients & healthy controls and correlate their levels with serum hTSH (highly sensitive Thyroid stimulating hormone) in a north Indian population.

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## MATERIALS & METHODS

This analytical, case control study was conducted in the Department of Biochemistry of a tertiary care hospital, Delhi, India. We enrolled 100 subjects after an informed written consent. This study had been approved by institutional ethics committee. Case group was formed by 50 patients (age group: 25-50 yrs) of recently diagnosed hypothyroidism selected from the hospital medical OPD with patients from different regions of northern India. Diagnostic criteria for hypothyroidism was (i) serum hTSH levels > 10 µIU/L, or (ii) serum hTSH levels between 6 to 10 µIU/L along with decreased serum FT3 &/or FT4 levels. Anti-TPO titres were negative in all patients. None of the patient had been started on treatment for hypothyroidism at the time of taking sample.

Exclusion Criteria: Patients with renal disease, rheumatologic disease, dermatological disease, hepatic disease, Diabetes Mellitus, alcoholics, & those on dietary supplements were excluded from the study.

Control group consisted of apparently healthy volunteers of comparable age group and gender (n=50). They visited the health care centre for comprehensive health check-up or were volunteers for blood donations. None of them had any history of intake of dietary supplements or thyroid disorder related drugs. All subjects underwent the same protocol.

### Assays

Fasting venous sample was collected under sterile conditions. Fasting sugar levels were also estimated using glucose-oxidase (GOD-POD) method to exclude patients with impaired glucose tolerance. Serum urea was estimated using urease-GLDH kinetic method and serum creatinine levels were measured using modified jaffe's kinetic method to rule out any renal pathology. All the mentioned biochemical tests were done using automated analyzer (CX series, Beckman Coulter, Inc. US). Serum hTSH (highly sensitive TSH) and intact-PTH levels were estimated immediately using chemiluminescence based immunoassay (Beckman Access II, Beckman Coulter, Inc., Fullerton, CA). i-PTH assay had a sensitivity of 1 pg/ml and an inter assay coefficient of variation (CV) of less than 6.5%; with the normal range of serum i-PTH as 10–65 pg/ml. Serum 25(OH) Vitamin D levels were assayed using ELISA (DRG kit, The USA) with a sensitivity of 1.5 ng/ml and an inter assay (CV) of less than 10.5%. We used chemiluminescence based technique for hTSH, free T3 and free T4 immunoassay (Beckman Access II, Beckman Coulter, Inc., Fullerton, CA). hTSH had sensitivity of 0.003 µIU/L and inter assay CV of less than 20%. All the analysis was performed in duplicates and average values used.

### Statistical analyses

All analysis was done using IBM SPSS software (Version 20.0, IBM SPSS, IL, USA). Group data are presented as mean values ± S.E.M. Quantitative data was assessed using independent sample student's t-test. An association between study variables was assessed using Pearson's correlation analysis. Regression analysis was done to determine the strength of association. A value of  $p < 0.05$  was considered statistically significant.

## RESULTS

The baseline characteristics of the two groups were comparable with respect to age and sex.

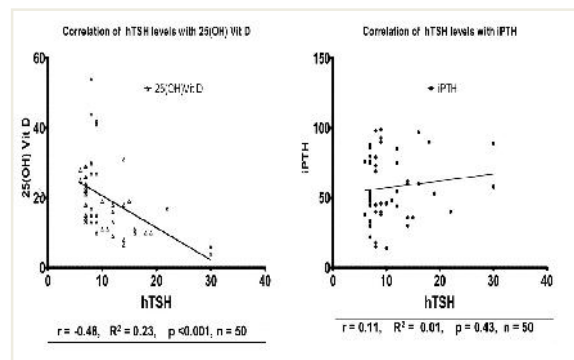
Biochemical analysis shown in Table: 1 revealed that serum hTSH levels were significantly higher in cases ( $10.72 \pm 0.77$  µIU/ml) as compared to controls ( $2.68 \pm 0.14$  µIU/ml). Mean levels of serum 25 (OH) vitamin D in hypothyroid patient group was ( $20.0 \pm 1.49$  ng/ml) compared to controls ( $27.7 \pm 1.34$  ng/ml). Serum i-PTH levels were found to be significantly higher in cases ( $57.6 \pm 3.30$  pg/ml) than controls ( $36.8 \pm 1.91$  pg/ml), see table 1.

**Table 1** Biochemical characteristics in study population

Parameters	Case (Mean ±S.E.M)	Controls (Mean ±S.E.M)	p value
Vitamin D (ng/ml)	20.0 ± 1.49	27.7 ± 1.34	0.001*
PTH (pg/ml)	57.6 ± 3.30	36.8 ± 1.91	0.001*
TSH (µIU/ml)	10.72 ± 0.77	2.68 ± 0.14	0.001*

\*p value < 0.05 is considered statistically significant. All values are expressed as

As observed in Figure: 1, Pearson's correlation analysis depicted a significant and negative correlation between levels of 25(OH) Vitamin D and hTSH ( $r = -0.48$ ,  $p < 0.001$ ). Most of the hypothyroid patients had serum 25(OH) Vitamin D levels below 20ng/ml. There was a positive correlation between serum hTSH and i-PTH levels, though it was not statistically significant ( $r = 0.11$ ,  $p = 0.43$ ).



**Figure 1** Correlation of serum hTSH levels with 25(OH) Vitamin D & i-PTH in hypothyroid subjects

hTSH- highly sensitive thyroid stimulating hormone  
iPTH- intact parathyroid hormone

When regression analysis was performed keeping hTSH as a dependent variable, i-PTH & 25(OH) Vitamin D levels were found to contribute to the risk of hypothyroidism. An assessment of standardized regression coefficient (Beta) revealed that, 25(OH) Vitamin D was a stronger predictor of hypothyroidism risk ( $\beta = 0.5$ ) compared to i-PTH as seen in table 2.

**Table 2** Regression analysis performed on hypothyroid subjects

Independent variable	Standardized Coefficient	Independent variable
25 (OH) Vitamin D	-0.392	<0.001*
I-PTH	0.305	<0.001*
Dependent variable : hTSH		

\*p value < 0.05 is considered statistically significant

## DISCUSSION

Low levels of 25(OH) Vitamin D is now a commonly accepted finding in north Indian population and overt deficiency has often been associated with a number of clinical disorders. We observed in this study that vitamin D does have a role to play in hypothyroidism as a significant correlation was found between serum hTSH levels and serum 25(OH) Vitamin D

levels even though a causal relationship could not be established. In this study, the hypothyroid patients had significantly lower levels of serum 25(OH) Vitamin D as compared to controls ( $p < 0.001$ ). One possible explanation for these reduced levels of 25(OH) Vitamin D in cases can be the sluggish intestines as seen in hypothyroidism and thus reduced absorption of Vitamin D. Since the primary source of vitamin D in body is its synthesis from cholesterol in skin with the help of sunlight, there seems to be other factors as well leading to its insufficient levels. A recent study suggested that Vitamin D deficiency may lead to Grave's disease and its deficiency has also been associated with auto-immune thyroid disorders and its protective role has been mentioned due to its immune regulatory effect (Rotondi and Chiovato, 2013; Goswami *et al.*, 2009). We observed, in this study, that 56% hypothyroid patients had 25(OH) Vitamin D levels below 20ng/ml whereas only 10% had sufficient levels. Healthy controls had higher levels of vitamin D but still the values were towards the lower end of the spectrum (Goswami *et al.*, 2000).

Serum i-PTH levels were higher in cases as compared to controls secondary to decreased Vitamin D levels ( $p < 0.001$ ). This increased i-PTH stimulates activity of 1-alpha-hydroxylase enzyme in kidney and ensures availability of active Vitamin D. So in individuals with poor bioavailability or already deficient levels of Vitamin D, the levels of active form may remain normal initially when 25(OH) Vitamin D levels tend to fall. This would maintain the levels of serum calcium and phosphate within normal range initially but gradually even the levels of active vitamin D may fall and worsen the symptoms associated with calcium homeostasis. Moreover, studies have suggested that the target cells are less responsive to the effects of i-PTH in hypothyroidism, and as a consequence the levels of active vitamin D remain low (Adams *et al.*, 1968). Studies have concluded that the requirement of 25(OH) Vitamin D for patients with thyroid diseases is probably higher than healthy population to control parathyroid hormone levels (Zhang and Naughton, 2010). The rise in levels of serum i-PTH in vitamin D deficient individuals is in accord with other studies (Goswami *et al.*, 2000).

We observed a negative correlation of serum 25(OH) Vitamin D levels with hTSH in hypothyroid patients on Pearson's correlation analysis ( $r = -0.48$ ,  $p < 0.001$ ) suggesting an inter relationship that exists between vitamin D insufficiency and hypothyroidism. It also states of a putative role of vitamin D as a potential modifiable risk factor for hypothyroidism. In order to function, vitamin D must bind to its receptor VDR which is found in several cell types including thyroid gland (Norman, 2006, 2008). Studies have shown that patients of autoimmune thyroid disease have several VDR polymorphisms that affect its expression and activation (Zaletel and Gaber, 2011). So probably vitamin D plays a role in maintaining a euthyroid state by interacting with its receptor in the thyroid gland.

On performing regression analysis, with hTSH as dependent variable and 25(OH) Vitamin D & i-PTH as independent variables, we observed that low levels of 25(OH) Vitamin D ( $\beta = -0.5$ ) is a stronger predictor for hypothyroidism as compared to i-PTH. Vitamin D is the only vitamin that acts through nuclear receptors (VDR) and thus, modulates gene expression at transcription level. Thyroid hormones also act through nuclear thyroid receptors (TR) and they both share the same hormone response elements within the nucleus. Studies

have suggested that even though they both form dimers with retinoic receptors (RXR) but show no interactions or dimerizations with each other. The cross talk is possible only when excess of one affects the dimerization of the other. So in hypothyroidism when the levels of thyroid hormones fall, TR levels rise to sequester RXR which prevents heterodimerization of VDR, thereby resulting in repression of active vitamin D induced transactivation (Pandya *et al.*, 1998). This suggests that vitamin D must be present in sufficient amounts for thyroid hormone mediated actions and vice versa. Moreover, vitamin D does interact with thyroid gland through its VDR and may affect the production of thyroid hormones. In vivo, supplementation of vitamin D has shown protective role and forestalled the development of autoimmune thyroiditis (Fournier *et al.*, 1990).

## CONCLUSIONS

To conclude, the results of the present study suggest that moderate to severe vitamin D deficiency is a risk factor for hypothyroidism. As vitamin D deficiency is highly prevalent in north Indian population, these findings may have some public health implications. Further, large scale clinical trials and prospective studies will be required to establish a cause and effect relation of vitamin D deficiency in pathogenesis of hypothyroidism. Supplementation of vitamin D in hypothyroidism may ameliorate its symptoms and prevent further deterioration of thyroid function, so, for the ease, safety and low cost of treating this modifiable risk factor we recommend its screening in all hypothyroid patients.

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