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# A study of serum vitamin D and calcium levels in subclinical hypothyroid patients

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## Abstract

**Objectives:** Hypothyroidism and vitamin D deficiency are one of the most common worldwide. Vitamin D is not only a vitamin but also a steroid hormone, which acts through VDR receptors .Mineral metabolism is frequently disturbed in thyroid dysfunctions. Hypothyroidism may be caused by immune or non- immune causes.

We aimed to examine the relationship between vitamin D and subclinical hypothyroidism and to clarify the relation between calcium and hypothyroid disease.

**Material and methods:** A hospital based cross sectional study consists of 109 cases of patients (74 females & 35 males) and 41 normal healthy controls (25 females & 16 males) meeting the selection criteria. For the quantitative measurement of serum TSH, T3, T4, total 25-OH vitamin D & ca in human serum using the VITROS ECi/ECiQ immunodiagnostic systems, the VITROS 3600 and the VITROS 5600 integrated system.

**Results:** A statistically significant negative correlation between serum vitamin D and TSH levels was noticed. Also statistically significant negative correlation between serum calcium and TSH levels was observed. There was a positive correlation between vitamin D and calcium levels.

**Conclusion:** our study indicated that vitamin D and calcium levels are significantly altered in hypothyroid patients. Thus monitoring of vitamin D & calcium in hypothyroid patient will be great benefit in improving clinical manifestation and can be treated appropriately.

**Keywords:** Hypothyroidism, TSH (thyroid stimulating hormone), vitamin D, VDR (vitamin D receptor), serum calcium levels

## Introduction

Thyroid disorders are amongst the most common endocrine Diseases in India (1). Hypothyroidism is defined as a deficiency of both T3 and T4 resulting in decreased thyroid activity. Biochemically decrease in T3 and T4 concentration leads to hyper secretion of pituitary TSH and an amplified increase in serum TSH levels [2]. Hypothyroidism is a progressive disorder presenting with different degrees of thyroid failure and metabolic consequence (3). Subclinical hypothyroidism is a milder form of hypothyroidism characterized by an elevated serum TSH level, but with a normal serum free thyroxin level. This milder form

of hypothyroidism is most commonly caused by Hashimoto's thyroiditis. The term subclinical hypothyroidism was first introduced in 1970s coincident with the introduction of serum thyrotropin (TSH) measurement (Jotideb Mukhopadhyay *et al*).

In western countries, hypothyroidism occurs in 0.3% to 0.4% of people, while subclinical hypothyroidism occurs in 4.3% to 8.5% of people. The mean annual incidence rate of hypothyroidism is up to 4 per 1000 women, 1 per 1000 men, 1 in 4000 inborn. Thyroid disorders are amongst the most common endocrine diseases in India. The total burden of thyroid disorders in India is 42 million. The prevalence and pattern of thyroid disorders depends on sex, age, ethnic and geographical factors and especially on iodine intake (4). The prevalence of hypothyroidism increases with age (5). Hypothyroidism is ten times more common in women than men (6). Decrease in T3 and T4 concentration leads to hyper secretion of pituitary TSH and an amplified increase in serum TSH levels. This is a key laboratory finding in diagnosis of hypothyroidism (7).Thyroid hormones perform a wide array of metabolic functions including regulation of lipids, carbohydrates, protein and electrolytes and mineral metabolism(8). Mineral metabolism like calcium, magnesium and phosphorous is frequently disturbed in thyroid dysfunctions(9).

Deficiency of thyroid hormones in early life leads to both delay in the development of bone and stippled appearance of epiphysial centres of ossification, this result possible dwarfism (10). Perhaps the most commonly known function of vitamin D is its role in keeping bones strong by helping body absorb calcium. However, vitamin D has other roles, such as regulating the immune system, and it may even play a role in keeping thyroid hormone functioning properly (11). Vitamin D is recognized as fat soluble vitamin or steroid pro hormone. Exposure to ultraviolet B light (290-320nm) are the main source of vitamin D (12).Classical endocrine pathway, vitamin D enters the circulation attached to a D-binding protein, is first hydroxylated in the liver to 25(OH) D and then in the kidney to form the active metabolite, 1,25 dihydroxyvitamin D [1,25-(OH)2 D] or calcitriol. Serum 25(OH) D, the most abundant circulating precursor of active vitamin D, is the most widely accepted indicator of vitamin D status and reflects combined contributions from cutaneous synthesis (13).

Serum 25(OH)D has a half –life of approximately two to three weeks, in contrast, 1,25-(OH)2D has a short circulating half life and is tightly regulated over a narrow range by parathyroid hormone, calcium and phosphate. Serum 1,25(OH)2D is not a good measure of vitamin D status since a decrease may not occur until vitamin D deficiency is severe. It was thought that one of two mechanisms may explain the low levels of vitamin D in patients with hypothyroidism, 1) the low levels of vitamin D may be due to poor absorption of vitamin D from the intestine or 2) the body may not activate vitamin D properly. Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors (14)

Much evidence suggests that low vitamin D levels may have a negative effect on thyroid function. The degree of vitamin D deficiency may link to the severity of hypothyroidism .The more severe the hypothyroidism the lower the vitamin D levels. The study was published in the November 2013 issue of the international journal of health sciences (10).Vitamin D deficiency has been shown to be associated with autoimmune diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), inflammatory bowel disease (IBD), multiple sclerosis (MS) and type 1 diabetes (TIDM), and that vitamin D supplementation prevents and /or development of these autoimmune diseases (15).However, for vitamin D to act,

it needs to bind a set of special receptors at those sites of action. These receptors are called vitamin D receptors (VDRs) and they can be found in almost all the organs of the body.VDRs themselves, are a subset of thyroid hormone receptors.VDRs are found on white blood cells including activated T and B cells as well as monocytes. By binding to these receptors on these immune cells, vitamin D can prevent them from triggering the autoimmune destruction of the cells of the thyroid gland. VDR polymorphism is common in those suffering from autoimmune thyroid disorder like Hashimoto thyroiditis. Polymorphism in VDRs means that the biological activity of vitamin D is reduced even when it binds to these receptors. When polymorphic VDRs are present in the thyroid gland, even normal level of vitamin D cannot produce same effects as it does on normal VDRs (brad chase).

So our study was undertaken to estimate the alterations or relationship in the levels of serum calcium and vitamin D in patients with subclinical hypothyroidism. We also explored the correlation between TSH levels and the serum calcium and vitamin D levels.

In hypothyroidism increased production of thyroid calcitonin can promotes the tubular reabsorption of phosphate and also favours the tubular excretion of calcium. Thyroid hormones probably stimulate bone resorption directly, thereby increasing serum calcium and phosphorous concentrations and also suppressing serum parathyroid hormone and 1, 25-dihyroxycholecalciferol (vitamin D3) concentrations (5).So in the hypothyroidism there is a depressed turnover due to impaired mobilization of calcium into the bone then leads to decrease the blood calcium level. Indian patients are different from western patients from bone mineral haemostasis point of view. On one hand, thyroid disorders are most common prevalent conditions and on the other hands, Indian studies focusing on the blood levels of calcium and phosphorous in thyroid disorders are sparse. The effect of thyroid hormones on minerals is not well established and the underlying mechanism is not well understood also (6).

**Materials and methods**

The study was conducted in the Department of Biochemistry of Shri Guru Ram Rai Institute of Medical and Health Sciences, SMI hospital, UK Dehradun. Over a period of 6 months from January 2016 to June 2016 150 samples was drawn comprising of 109cases (35 males, 74 females) and 41 control patients (16 males and 25 females).

1. **Study design:-**
   The study was carried out under two heading-
   I. Cross sectional study
   II. Observational study
2. **Source of data:-**
   Case group- 109
   Control group- 41

The sample was chosen from among the patient who visited Outpatient department of Medicine of SMI Hospital during the reference period. The sample of 41 healthy persons was drawn from among the persons catering in various units of Shri Mahant Indresh Hospital. Subjects were recruited according to simple random sampling method that met the selection criteria. The age group of all the samples persons from 20 to 75 years was taken. Both the gender was taken.

3. **Inclusion criteria:**
   Known cases of subclinical hypothyroidism patients aged between 20 to 75 years with TSH level > 4μIU/ml and total T3<1.08nmol/L, total T4 (<59nmol/L) irrespective of their treatment status taken as inclusion criteria.

4. **Exclusion criteria:**
   Patients with history of hepatic disease, renal disease, bone disease, alcoholism, medical conditions or any medications that might affect serum calcium and vitamin D concentrations were excluded from the study.

5. **Study tools:**
   All cases included in this study were subjected to the followings:-
   I. Complete history taking.
   II. Complete clinical examination.
   III. Laboratory investigations.

6. **Collection of blood samples:**
   For both the case and control groups, samples were collected into plain vacutainers, under quality control procedures.5 ml fasting blood samples were drawn from hypothyroid patients and healthy volunteers. Blood samples were allowed to clot for 10 to 15 minutes and then immediately centrifuged at 3000 rpm for 10 min. Serum were separated from the clotted blood.

7. **Analysis of sample:**
   Serum TSH was measured by the VITROS ECi/ECiQ immunodiagnostic system, the VITROS 3600 immunodiagnostic system and the VITROS 5600. An immunometric immunoassay technique was used. Serum $T_3$ & $T_4$ were estimated by using the VITROS FT3 or FT4 reagent pack and the VITROS FT3 OR FT4 calibrators on the VITROS ECi/ECiQ immunodiagnostic systems, the VITROS 3600 immunodiagnostic system & the VITROS 5600 integrated system, a direct labelled antibody, competitive immunoassay technique is used. Vitamin D total test was performed by using the VITROS 25-OH vitamin D total reagent pack and the VITROS 25- OH vitamin D total calibrators on the VITROS ECi/ECiQ immunodiagnostic systems, the VITROS 3600 immunodiagnostic system and the VITROS 5600 integrated system. A competitive immunoassay technique is used. Serum total calcium level was estimated by Arsenazo III method.

8. **Statistical Analysis:**
   Statistical Analysis was done on Microsoft excel software. The Mean, Standard deviation (SD) and Standard error for all the variables were calculated. Continuous parameters were expressed as Mean±SD±SE. The correlation between different parameters like vitamin D, calcium and TSH was established by Pearson coefficient of correlation(r).P value <0.05 was considered statistically significant.

**Results**

The study was done in the department of biochemistry of SGRRIM&HS and SMI Hospital. Total of 150 subjects out of which 109 cases and 41 control groups. The case groups are included in this study were from outpatient department of medicine of SMI Hospital. The details of the patients were recorded in Performa.

**Table 1:**

| Parameter | Control | Case |
|---|---|---|
| TSH | 4μIU /L | >4μIU/L |
| T3 | 4.26-8.10pmol/L | <4pmol/L |
| T4 | 10-28.2pmol/L | <10pmol/L |
| Vitamin D | 20-50ng/ml | <20ng/ml |
| Calcium | 9-11mg/dl | < 9mg/dl |

The values obtained on analyzing specimens collected from patients and control groups are tabulated. The mean values, standard deviation and standard error also have been calculated for comparative study of patients and controls. The values of patients and control groups are also graphically represented for comparison at a glance. The

graphs were plotted using mean values of all the study parameters.

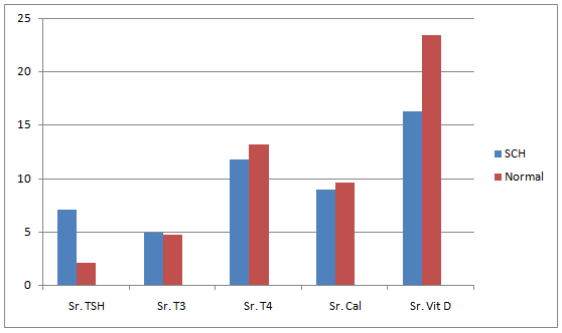


**Figure 1:**

On comparing the vitamin D and calcium levels in female control and case patients, we found that vitamin D insufficiency (<20ng/ml) was prevalent among subclinical hypothyroid female patients. There is a negative correlation between vitamin D and TSH, represented by r= -14.179, p value is <0.00001 (significant at p<0.05). In our study we noticed that serum TSH level was significantly higher in hypothyroid patients than controls (t-test= -11.064, p<0.00001). There was also significant negative correlations between serum calcium levels and TSH (r= -15.483, p value<0.00001).There was significant positive correlations between vitamin D and calcium levels (r= 2.455, p value is 0.007044).

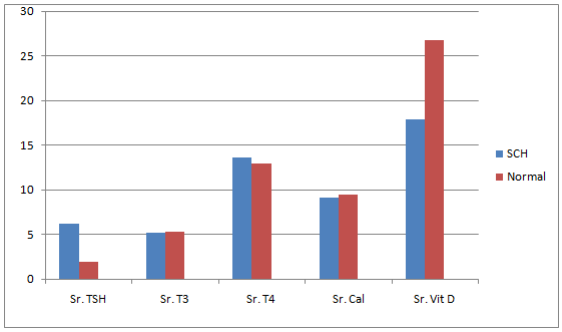


**Figure 2:**

By using t-test to compare between subclinical hypothyroid male patients and controls, serum TSH levels was significantly higher in male cases than in controls (t-test= -10.874, p value<0.05).vitamin D levels was significantly lower in subclinical hypothyroid patients than in controls (t=13.760, p<0.05).there was a significant negative correlations between TSH levels and vitamin D levels (r= -13.793, p<0.05). Serum calcium levels had a significant negative correlations with serum TSH (r= -15.361, p value<0.00001).

**Table 2: Comparison of analytes between control (25) and subclinical hypothyroid female(74) patients.**

| Tests | Control(mean±SD±SE) | SCH(mean±SD±SE) | P value |
|---|---|---|---|
| **TSH** | 6.19±2.07±0.41 | 7.13±3.40±0.39 | p<0.05 |
| **T3** | 5.14±1.0±0.2 | 4.98±1.46±0.16 | p= 0.172329 |
| **T4** | 13.69±4.29±0.8 | 11.77±3.51±0.4 | P<0.05 |
| **Sr Ca** | 9.14±0.46±0.33 | 9.03±0.49±0.12 | P<0.05 |
| **VitaminD** | 17.93±3.98±0.13 | 16.25±5.61±0.78 | P<0.05 |

**Table 3: Comparison of analytes between control and subclinical hypothyroid male patients.**

| Tests | Control(mean±SD±SE) | SCH(mean±SD±SE) | P value |
|---|---|---|---|
| **TSH** | 1.89±1.01±0.25 | 2.14±0.94±0.15 | p<0.05 |
| **T3** | 5.30±0.85±0.21 | 4.80±0.51±0.08 | p<0.00001 |
| **T4** | 12.96±2.40±0.6 | 13.24±3.36±0.56 | p=0.454223 |
| **Sr Ca** | 9.53±0.37±0.09 | 9.67±0.43±0.12 | p<0.05 |
| **Vit D** | 26.79±4.49±2.11 | 23.41±3.99±1.17 | p<0.05 |

**Table 4: Comparison of analytes between subclinical hypothyroid female patients (74) and subclinical hypothyroid male patients (35).**

| Test | SCH females | SCH males | P value |
|---|---|---|---|
| TSH | 7.13±3.40±0.39 | 6.19±2.07±0.41 | p=0.000115 |
| T3 | 4.98±1.46±0.16 | 5.14±1.0±0.2 | p <0.00001 |
| T4 | 11.77±3.71±0.4 | 13.69±4.29±0.8 | p = 0.004158 |
| Calcium | 9.03±0.49±0.12 | 9.14±0.46±0.33 | p<0.05 |
| Vitamin D | 16.25±5.61±0.78 | 17.93±3.98±0.13 | p=0.120198 |

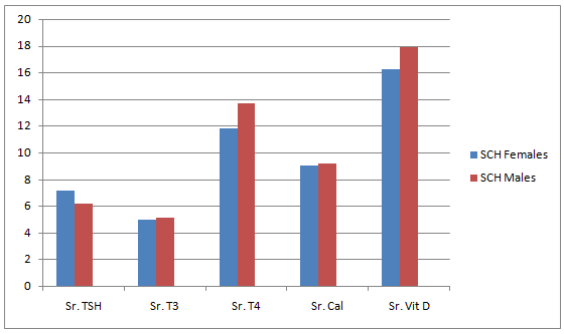


**Figure 3:**

When serum TSH levels in subclinical hypothyroid patients were compared according to sex, we noticed a significant difference between males and females patients (t = -3.684, p value is 0.000115). Serum T3 and T4 levels also lower in females than in male subclinical hypothyroid patients. On comparing the vitamin D levels according to the sex distribution, we observed a non -significant difference between males and females patients (t= -1.174, p>0.05). Concerning serum calcium levels, it was noticed that calcium levels was slightly lower in female patients than in males.

**Discussion**

Thyroid diseases are among the most common endocrine abnormalities and AITDs are perhaps the most prevalent autoimmune diseases (16).Thyroid hormone is a central regulator of body hemodynamics, thermoregulation and metabolism. Thyroid hormone affects the glomerular filtration rate and blood flow and has direct effect on Calcium resorption (7).

Vitamin D is not just a vitamin, but also a hormone and it is also an important immunomodulator. The elevated TSH levels as observed in hypothyroidism causes metabolic changes and functions of specific organs which leads to bone disorders and other endocrine related disorders(17).Wei-young Lin, Lei Wan, Chang-Hai Tsai *et al* states that for many years vitamin D status was defined simply by whether or not the patients had symptoms of the bone disease i.e., rickets in children and osteomalacia in adults. Of late, vitamin D deficiency has been associated with increased risk of cancer, cardiovascular disease and many autoimmune diseases like type1 diabetes mellitus, rheumatoid arthritis, multiple sclerosis, autoimmune thyroid disease, Crohn's disease etc.

According to the Shaye kivity, Nancy Agmon-Levin(2016) *et al* at the present time vitamin D levels above 30ng/ml are considered sufficient and confer protection from bone disease, whereas lower levels(vitamin D insufficiency) induce elevation of parathyroid hormone and are associated with other hazardous systemic effects. Very low levels of this hormone, defined as 'vitamin D deficiency', were recently suggested to be related to even worse outcomes.

To examine an involvement of vitamin D and Calcium levels in the hypothyroidism, we evaluated the vitamin D and Calcium status among patients with hypothyroidism compared to healthy controls who did not complain from hypothyroidism or any thyroid diseases. Our study demonstrated

significant low levels of serum vitamin D in cases than controls. In the present study the serum calcium levels were slightly low in cases of hypothyroidism as compared to healthy controls.

Several studies have established a relationship between low vitamin D and autoimmune thyroid disorders. Our results revealed decreased serum 25(OH) vitamin D levels in female controls than those of male controls and patients.

Moreover, in contrast to our results ($p>0.05$), Sedrani, AL-Jurayyan et al, Fida, Naeem et al, stated that vitamin D serum levels are significantly more decreased in females than males. Hashemipour et al studied the prevalence of Vit D in Tehran and found non- significant differences between males and females without association between Vit D and sunlight exposure. This study goes favour in our study. According to our study, serum vitamin D levels recorded a non-significant difference between male and female patients.

Byron Richards (2008) studied the effect of Vitamin D deficiency on thyroid gland in experimental study; he reported that a lack of vitamin D contributed to the possibility of low thyroid hormones. One of the two mechanisms may explain the low levels of vitamin D in patients with hypothyroidism. First, the low levels of vitamin D may be due to poor absorption of vitamin D from intestine. Second, the body may not activate vitamin D properly (2). Since vitamin D is absorbed in the small intestine, a leaky and inflamed GI tract- which is extremely common in people with low thyroid function reduces the absorption of vitamin D (Chris Kresser,Aug 4,2010).

Jesse, Sulzer (2011) reported a significant negative correlation between serum vitamin D and circulating levels of TSH, meaning that low levels of vitamin D were associated with worsened thyroid function in thyroid patients. This study also goes in favour of our study.

In our study we set TSH levels >4 μ IU/ml, T4 < 10 p mol/l, T3 <4pmol/l, vitamin D< 20ng/ml and Ca level < 9mg/dl related to hypothyroidism. In our study we observed that the vitamin D levels (Mean ±SD=23.41±3.99 in male & 16.25±5.61 in female) is lower in cases compared to controls. There was a negative correlation between vitamin D and TSH levels. When we compared female controls and female hypothyroid patients, we observed that there was a significant negative correlation between TSH levels and vitamin D Levels ($p<0.00001$).That was also seen in male controls and male hypothyroid patients ($p<0.00001$).

Another plausible explanation according to Shaye kivity, Nancy Agmon-Levin *et al*(Jan 2011) is that accelerated bone turnover in patients with hyperthyroidism may lead to high calcium levels and a negative feedback on parathyroid hormone and $1,25(OH)_2D_3$ synthesis. Therefore, it seems that a primary role for vitamin D deficiency in the pathogenesis of thyroid disease should be considered.

Shipla Hasija Bhardwaj *et al* (may 2014) has also observed a negative correlation of serum 25(OH) vitamin D levels with high TSH in hypothyroid patients on Pearson's correlation analysis ($r= -0.48$, $p<0.001$) suggesting an interrelationship 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). Zaletel nd Gaber's (2011) study has shown that patients of autoimmune thyroid diseases have several VDR polymorphisms that affect its expression activation. This study also goes in favour of our study ($p<0.05$).

Another study which is explained by Dong Yeob Shin, Kwang Joon Kim *et al* states that

the most effects of vitamin D are mediated via vitamin $D_3$ receptor (VDR).The immune modulator properties of vitamin D are ascribed to its effect on T and B lymphocytes, all of which harbour VDRs. Low vitamin D increase the prevalence of AITDs may be related to vitamin D deficiency, whose prevalence is also rising.

We also observed a non-significant difference between the male hypothyroid patients and female hypothyroid patients. The vitamin D levels is lower in female patients (Mean±SD=16.25±5.61) than males (Mean±SD=23.41±3.99).In the developing country like India females generally suffer from hypovitaminosis, in which vitamin deficiency is more frequently seen (Amal Mackway *et al).* Gulab Kanwar and Monika shekhawat *et al* studied that India has a noteworthy burden of both hypothyroidism and hypovitaminosis. Females are more prone to vitamin D deficiency due to their life style, cultural practices and excessive use of cosmetics.

Our study also indicates that association between calcium levels and subclinical hypothyroidism. We observed a significant low levels of serum calcium (Mean±SD= 9.67±0.43 in males & 9.03±0.49 in females) in cases than controls. There was a significant negative correlation between TSH and serum calcium levels ($p<0.00001$).

In hypothyroidism there is a depressed turnover due to impaired mobilization of calcium into the bone that leads to decrease blood calcium level. In hypothyroidism, there is also an increased production of thyroid calcitonin which promotes the tubular reabsorption of phosphate and favours the tubular excretion of calcium (35).

Roopa M *et al* & Jaskiran K *et al* studied changes in electrolyte profile in patient of hypothyroidism. There was a significant negative correlation between TSH and calcium level. Thyroxin normally regulates blood calcium level by releasing calcium from cells, by decreasing thyroxin level in blood, less thyroxin enters the cells and less calcium is released leading to hypocalcaemia.

Kavitha MM *et al* (2014) also agrees with our study, reveals a significant decrease ($p<o.ooo1$) in serum calcium levels in hypothyroid patients. The significant decrease in mean serum calcium level in hypothyroid patients seen in the present study is consistent with the studies conducted by Ala eldin *et al* and studies carried out by B. Suneel *et al*.

Anuradha A *et al.*2015 states that Usually hypocalcaemia is associated with hypothyroidism because low levels of PTH and low levels of calcitriol cause decreased absorption of calcium from intestine & increased tubular excretion of calcium.

Our study was further confirmed by the study done by Christoph Schwarza,*et al* who reported that there was a significant correlation between calcium and serum TSH,$T_3$ and $T_4$ in hypothyroid patients. In our study we also observed a significant difference in serum calcium levels between male and female patients. Female had lower calcium level (Mean±SD=9.03±0.49) than the male patients (9.67±0.43) with p-value< 0.00001.

## Conclusion

Our study demonstrated that hypothyroid patients also suffered from hypovitaminosis D with hypocalcaemia. Moreover, the positive significant correlation between each of serum vitamin D and calcium with thyroid hormones and that negative significant correlation with TSH levels, suggested that deficiency of serum vitamin D and calcium levels were significantly associated with degree and severity of the hypothyroidism which encourage the advisability of vitamin D supplementation. Therefore monitoring of serum levels of vitamin D & calcium during the follow up of hypothyroid patients will be great benefit.

**Limitations and recommendation**

The limitations of this study could be cut down in three points; first, the small number of subjects, second, confined in its ability to conclude that vitamin D status is directly related to the pathogenesis of hypothyroidism; third, we have to measure the parathyroid hormone (PTH), calcitonin level and analyze its effect on vitamin D and hypothyroid disease.

Therefore, the direct role of vitamin D in those patients with thyroid problem should be examined by further prospective clinical studies and examine the effect of the treatment of vitamin D and PTH on hypothyroidism. Screening for vitamin D and calcium levels is recommended for all hypothyroid patients. Moreover, supplementary vitamin D and calcium are recommended to patients with hypothyroidism.

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