



ORIGINAL RESEARCH PAPER

Biochemistry

STUDY OF SERUM LEVELS OF VITAMIN D AND CALCIUM IN PRIMARY HYPOTHYROID PATIENTS.

KEY WORDS: Vitamin D , Calcium , free T3 , free T4 , Hypothyroidism

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BACKGROUND Hypothyroidism is one of the most common endocrine disorders in whole world.¹ In humans primary hypothyroidism is a grossly prevalent thyroid disorder with preponderance in females. On the other hand vitamin D deficiency is a global health problem.² Even in Indian population this sunshine vitamin is reported to be deficient, despite the abundance of sunlight in this country. In addition, it is thought that vitamin D may play a role in the regulation of the immune system because vitamin D receptors are detected on cells of the immune system.³ The high prevalence of hypothyroidism and vitamin D deficiency in the Indian population provides a unique opportunity to assess the association between these two variables.

AIM & OBJECTIVE To study the blood levels of vitamin D and calcium in patients with primary hypothyroidism and create awareness among general hypothyroid subjects regarding the need as well as importance of blood vitamin D & calcium estimation over regular intervals to avoid the incidence of vitamin D and calcium deficiency.

MATERIAL & METHODS

Study Design:- The current study is a Comparative Cross Sectional Study in which hypothyroidism cases were compared with healthy subjects.

Study Period:- August 2019 to January 2020.

Inclusion Criteria :- Subjects who were diagnosed with primary hypothyroidism were enrolled in case group of this study. All patients suffering from hypothyroidism were diagnosed and confirmed by the physician based on free T3 , free T4 and TSH levels of the patients.

Exclusion Criteria :- Patients with secondary hypothyroidism post radioiodine hypothyroidism, hepatic or renal dysfunction, on anti-epileptic medications or vitamin D supplementation were excluded from the study.

Study subjects :- We conducted a study on the blood samples of those patients who were advised and referred to the CCL of IIMS&R medical college for thyroid profile (free T3, free T4 & TSH) tests and then diagnosed with primary hypothyroidism. So accordingly in this study we selected 75 subjects who were recently diagnosed with primary hypothyroidism in medicine OPD of IIMS&R Jalna medical college and these study subjects were categorised in Group I. We analysed their serum samples for the vitamin D & calcium and their respective levels were compared with vitamin D & calcium serum values of another 75 healthy subjects who were categorised in Group II.

So we had two groups ; first group was labelled as case group which consisted of 75 primary hypothyroid patients ageing less than 50 years and second was another comparable group comprising of 75 healthy subjects ageing less than 50 years which we labelled as control group. The objectives of the study were explained to all eligible subjects. Ethical approval for undertaking the study was taken from ethical committee of the IIMS&R medical college as well as due informed consent of all subjects included in the study was obtained for their involvement in study groups and for venipuncture.

Method of sample analysis:- Serum free T3 , free T4 , TSH and vitamin D estimations were done in the central clinical laboratory on cobas e411 biochemistry immunoassay analyser which used the principle of electrochemiluminescence for estimating these hormones. On the other hand serum total calcium estimation was done on erba EM 200 autoanalyser by using ortho-cresolphthalein complexone method (OCPC).

Method of statistical analysis:- The statistical analysis was performed using Microsoft Excel Software. The statistical data was systematically analysed and was represented in form of mean + S.D. Unpaired t-test was used to compare between two means of all parametric continuous variable and p-value < 0.05 was considered as statistically significant.

Results :- The mean levels of serum 25(OH) vitamin D in control group was 37.68 ng/ml while vitamin D values in hypothyroid group was found to be 17.83 ng/ml. When t-test was applied to compare the mean levels between these two study group it was found that 25(OH) vit D levels were significantly lower in the hypothyroid group (P=0.001) as compared to the levels in healthy group.

Conclusion :- This study reported significant decrease in the serum levels of vitamin D as well as calcium in the hypothyroid subjects enrolled in our study where as there was no abnormal alteration in the serum levels of vitamin D and calcium in healthy group.

ABSTRACT

INTRODUCTION :

Hypothyroidism is one of the most common endocrine disorders in whole world.¹ Primary hypothyroidism is a very common thyroid disease, whose prevalence is 0.5–2.0% among women and around 0.2% among men.² The prevalence of hypothyroidism in India accounts to 10-11%.³ Along with it the prevalence of subclinical hypothyroidism has been estimated to be 9% among Indian population.⁴ According to the study done by Orlova M et al, recently the number of hypothyroid patients with autoimmune diseases have also been increased by 2.1%.³

On the other hand vitamin D deficiency is a global health problem.⁵ Even in Indian population this sunshine vitamin is reported to be deficient, despite the abundance of sunlight in this country.⁶ In recent years, however, vit-D has been reported to have a role in the incidence of autoimmune diseases, heart diseases, cancer, inflammatory bowel diseases, diabetes, and rheumatologic diseases.⁷ In addition, it is thought that vit-D may play a role in the regulation of the immune system because vit-D receptors are detected on cells of the immune system.⁸ The high prevalence of hypothyroidism and vitamin D deficiency in the Indian

population provides a unique opportunity to assess the association between these two variables.³ There has been controversies between the studies on relation between vitamin D deficiency and hypothyroidism which in turn urged us to study this interesting context of hypothyroidism and vitamin D deficiency.⁹

AIM OF THE STUDY :- To study the levels of vitamin D and calcium in patients with primary hypothyroidism.

OBJECTIVE OF THE STUDY :- To make the general hypothyroid subjects aware of the need as well as importance of evaluating vitamin D & calcium blood levels at regular intervals in the management of hypothyroidism so as to prevent collateral deficiency of vitamin D and calcium in hypothyroidism.

MATERIAL & METHODS :-

Study Design :- The current study is a Comparative Cross Sectional Study in which primary hypothyroid Subjects were compared with healthy subjects.

Study Period :- August 2019 to January 2020.

Inclusion Criteria :-

Subjects having decreased serum free T3 and free T4 levels associated with increased TSH levels were diagnosed with primary hypothyroidism and such subjects were enrolled in case group of this study.

All patients suffering from hypothyroidism were diagnosed and confirmed by the physician based on free T3 , freeT4 and TSH levels of the patients.

Exclusion Criteria :-

Patients with secondary hypothyroidism, post radioiodine hypothyroidism, hepatic or renal dysfunction, on anti-epileptic medications or vitamin D supplementation were excluded from the study.

Study subjects :-

We conducted a study on the blood samples of those patients who were advised and referred to our CCL of IIMS&R medical college for thyroid profile (free T3, freeT4 & TSH) tests and then diagnosed with primary hypothyroidism in the medicine OPD of our medical college.

So accordingly in this study we selected 75 subjects who were recently diagnosed with primary hypothyroidism in medicine OPD of IIMS&R Jalna medical college and these study subjects were categorised in Group I. We analysed their serum samples for the vitamin D & calcium and their respective levels were compared with vitamin D & calcium serum values of another 75 healthy subjects who were categorised in Group II.

So we had two groups ; first group which consisted of 75 primary hypothyroid patients ageing less than 50 years and second was another comparable group comprising of 75 healthy subjects ageing less than 50 years. The objectives of the study were explained to all eligible subjects. Ethical approval for undertaking the study was taken from ethical committee of the IIMS&R medical college as well as due informed consent of all subjects included in the study was obtained for their involvement in study groups and for venipuncture.

Method Of Sample Analysis:

Vitamin D estimation was Outsourced to laboratory in Hedgwar hospital where its estimation was done on cobas e411 biochemistry immunoassay analyser. The method used for vitamin D estimation was electrochemiluminescence in which 25-hydroxy vitamin D was estimated instead of 1-25-dihydroxyvitamin D. 25-hydroxy vitamin D was preferred

inspite of being an inactive precursor as it is present in higher concentration in the blood and is having longer half life when compared to its active form 1-25-dihydroxyvitamin D.

We took the reference from endocrine society clinical practice guidelines¹⁰ according to which **vitamin D deficiency** was labelled as levels **below 20 ng/ml** & vitamin D levels **between 20-30 ng/ml** was labelled as **vitamin D insufficient** while levels **more than 30 ng/ml** was considered as state of **vitamin D sufficiency**.

Same method of electrochemiluminescence was also used for estimation of thyroid hormones which are free T3 , free T4 & TSH.

The following serum thyroid hormone values were considered as normal according to the kit insert information sheet:

- Normal free T3 → 2.4-4.2 pg/ml
- Normal free T4 → 0.7-1.4 ng/dl
- Normal TSH → 0.34-4.25 µIU/ml

On the other hand serum total calcium estimation was done on erba EM 200 autoanalyser by using endpoint Ortho-cresolphthalein complexone method (OCPC) in central clinical laboratory. This method is based on the reaction between OCPC and calcium ions in order to form purple coloured complex.

According to the medscape¹¹ reference the serum total calcium levels between 9 – 10.5 mg/dl was considered as normal where as the calcium levels below 8.8 mg/dl were labelled as calcium deficiency in adults.

Biochemical Analyte	Normal Levels	Deficiency Level Cutoff
Vitamin D	> 30 ng/dl	Vitamin D Deficiency
		< 20 ng/dl
Total Calcium	9 – 10.5 mg/dl	Vitamin D Insuficiency
		20 - 30 ng/dl
free T3	2.4 - 4.2 pg/ml	Calcium Deficiency
		< 8.8 mg/dl
free T4	0.7 - 1.4 ng/dl	Hypothyroidism
		< 2.4 pg/ml
TSH	0.34 - 4.25 µIU/ml	< 0.7 ng/dl
		> 4.25 µIU/ml

Table : 1

METHOD OF STATISTICAL ANALYSIS

The statistical analysis was performed using Microsoft Excel software. All the statistical data was systematically analysed and was represented in form of mean±S.D.

Unpaired t-test was used to compare between two means of all parametric continuous variable and p-value < 0.05 was considered statistically significant.

RESULTS

The data parameters are characteristically tabularised in table number 2.

The mean levels of serum 25(OH) Vitamin D in Group II was 37.68 ng/ml while vitamin D values in hypothyroid group was found to be 17.83 ng/ml.

When t-test was applied to compare the levels between two study group it was found that 25(OH) vit D levels were significantly lower in the hypothyroid case group (p = 0.001) as compared to the levels in healthy control group.

However in the hypothyroid group, when we compared the levels of 25(OH) vit D between the male and female patients insignificant difference was observed which was statistically proven by t and p values as p = 0.98.

Parameters	Control Group (Healthy Subjects)	Case Group (Hypothyroid Subjects)	p-value	Statistical Significance
	Mean ± SD	Mean ± SD		
Age	42.1 ± 6.35	39.7 ± 7.18	0.017	Non Significant
Gender	18 F (50%) 32 M (50%)	30 F (60%) 20 M (40%)	0.029 (Case Group)	Non Significant
Serum 25(OH)Vit D (ng/ml)	37.68 ± 4.24	17.83 ± 2.86	0.001	Significant
Serum Calcium (mg/dl)	9.47 ± 0.29	7.51 ± 0.42	0.001	Significant
Serum TSH (µIU/ml)	3.16 ± 0.39	8.37 ± 2.94	0.001	Significant
Serum FT3 (pg/ml)	2.13 ± 0.62	1.81 ± 0.30	0.002	Significant
Serum FT4 (ng/ml)	1.20 ± 0.17	0.5 ± 0.11	0.001	Significant

Table :2

DISCUSSION :

In Indian subcontinent it has been recently concluded in the study done by Rittu.G et al that the prevalence of vitamin D deficiency is witnessed in 70%-90% of the general population in the country and hence needs considerable attention regarding this subject of vitamin D deficiency.¹² It has also been witnessed in many other research studies that vitamin D may play a role in many biochemical mechanisms in addition to bone and calcium metabolism.¹³ Interestingly, it has newly been shown that vitamin D has potent immunomodulatory effects and plays important roles in the pathogenesis of autoimmune diseases. This association of vitamin D with autoimmunity was confirmed and established by researchers like Smolders J et al & Naderi et al in which they found that the vitamin D receptor (VDR) gene polymorphisms is present in many autoimmune diseases like DM-I , IBD & Multiple sclerosis.^{14,15} Because of these interesting newly found vitamin D properties and gross prevalence of vitamin D deficiency in India we were inspired to undertake this research study in which we found that the serum levels of vitamin D was significantly lower in primary hypothyroid group as compared to their healthy counterpart. Similar findings were reported in a study conducted by Vikram Sharma et al in which they attributed this effect of vitamin D to its property of immunomodulation.¹⁶ Due to immunomodulatory nature of vitamin D, hypothyroidism linked with vitamin D deficiency may be mainly due to autoimmune thyroid disorder i.e Hashimoto's thyroiditis. Hashimoto's thyroiditis is predominantly a disease of cell-mediated immunity that is manifested by a genetic defect in suppressor T-cell function.¹⁷ In Hashimoto's thyroiditis, the autoimmune process may be suppressed at various stages by 1,25(OH)2D. At first, vitamin D might suppress dendritic cell-dependent T cell activation, then, it might decrease proliferation of Th1 cells and the synthesis of Th 1 cell cytokines such as IFN γ .¹⁷ Furthermore, after being activated by T cells and B cells' ongoing proliferation may be suppressed and B cell apoptosis may be induced by 1,25(OH)2D. In this way, vitamin D might decrease autoantibodies that react with thyroid antigens.^{18,19} We also compared hypothyroid subjects serum calcium levels with the healthy group and we found that serum calcium levels were significantly decreased in the primary hypothyroid group. Our this finding was consistent with the other research study conducted by Heaney RP in which he attributed the decrease in serum calcium levels to the vitamin D deficiency. Another study done by Vikram Sharma et al found that in hypothyroid patient there was decrease in blood calcium levels because of impaired mobilization of calcium into bones.¹⁶ Similar findings were also reported by Shridevi et al

and Murgod R et al in which they observed that disturbance of calcium homeostasis was a common finding in subjects with thyroid dysfunction. They gave the justification of their finding that thyroxine normally regulates blood calcium levels by releasing calcium extracellularly. So in hypothyroidism as their is decreased thyroxine in the bloodstream less thyroxine will enter into the cells which in turn will cause decreased calcium release from the cells and ultimately it will lead to decrease in the levels of calcium in blood.^{21,22}

CONCLUSION :

This study reported significant decrease in the serum levels of vitamin D as well as calcium in the hypothyroid subjects enrolled in our study where as there was no abnormal alteration in the serum levels of vitamin D and calcium in healthy group. Thus it can be concluded that vitamin D deficiency may have a potential role in the development of hypothyroidism and hence these hypothyroid patients can be recommended to get their blood vitamin D and calcium levels estimated at regular intervals so as to prevent the suffering from vitamin D and calcium deficiency.

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