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Role of vitamin D in the prevalence of hypothyroidism

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Abstract
Introduction: The pandemic of vitamin D has been reaching new heights ever year. Over a billion people are affected by vitamin D deficiency or VDD. Most countries have reported to have 30-50% of the population with VDD. Meanwhile hypothyroidism in India is about 30%. There are many reasons for the occurrence of hypothyroidism, Out of which the deficiency of vitamin D may be one.
Sampling: CONTROLS: - A total of 30 serum samples of healthy people were taken who had normal levels of all thyroid parameters and normal levels of vitamin D.
TEST: - A total of 30 serum samples of patients suffering from hypothyroidism who also had deficient amounts of vitamin D in the serum were collected.
Methodology: Serum samples of patients and controls were taken by centrifugation and removal of formed elements of the blood. This serum is then put in a machine- Cobas e 601 in which many hormones and vitamins can be measured. This machine works on the principle of chemiluminescence. This gives the approximate value of vitamin D and thyroid levels which can be used for study
Results: A total of 15 women of ages 40-60 years and 15 men of 40-60 years were found to have a negative correlation between vitamin D and hypothyroidism levels in the serum. It was also observed that this is not a sex related disorder. Screening for vitamin D deficiency is recommended for hypothyroidism patients.

Keywords: vitamin D, VDD, hormone, hypothyroidism

Introduction
Vitamins are organic molecules which make essential micronutrients that an organism requires in exceedingly small quantities for the suitable operation of its metabolism. Vitamin D or calciferol or sunshine vitamin is a water insoluble vitamin is naturally found in very few of foods [1]. Vitamin D is found in 5 forms or vitamers, out of which only vitamin D is available in our body and the rest are obtained from plants. Exposure to UV-B light (290-320 nm) is the main source of vitamin D. From the endocrine pathway, the sunshine vitamin enters the blood stream bound to vitamin D receptor protein (VDR), is primarily hydroxylated in the liver to 25-hydroxyvitamin D and then it is transported to the kidneys and then in the kidneys for the formation of the active metabolite, 1,25 dihydroxy vitamin D or 1,25(OH)D or calcitriol. Serum 25(OH)D has a half-life of approximately 2-3 weeks greater than 1,25(OH)D of a mere 15 hours which is tightly controlled over a narrow range by calcium, phosphate, and parathyroid hormone. This is the reason why 25(OH)D levels are preferred over 1,25(OH)D levels which do not show decrease until the vitamin D deficiency is severe [2].
The thyroid gland is an endocrine gland which lies in the anterior part of the neck on the windpipe consisting of two lobes. It produces two hormones, triiodothyronine (T3), and thyroxine (T4) is more active hormone, directly affecting the tissues. Thyroid hormones affect metabolism, brain development, menstrual cycles and others. Iodine is essential for the production of T3 and T4. TSH is released from the anterior part of the pituitary gland. Low thyroid hormone levels result in the pituitary releasing more TSH. While high hormone levels trigger the pituitary by dropping TSH. High T3 and T4 levels indicate hyperthyroidism which results in high metabolism showing low levels of TSH while low levels of T3 and T4 indicate hypothyroidism which also shows high levels of TSH in the blood serum.
For this study, the level for normal levels of TSH for adults was taken as 0.8 – 1.8 ng/ml. for T4, the normal levels was considered to be 6.0 – 12.23 ug/dl and for T3, results were noted to be normal when the reference was in the range of 0.35 – 9.0 uIU/ml.
Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in the Vitamin D receptor was shown to predispose people to autoimmune thyroid disease including Graves' disease and Hashimoto's thyroiditis [2].

Aims and Objectives
The aim of this study is to observe and summarise recent data on possible association of vitamin D with hypothyroidism.

Materials and Methods
The present study was done in St Francis college for Women in collaboration with KIMS, a corporate Hospital. The blood samples were collected from different patients and controls and centrifuged and serum was collected. This collected serum was then put in Roche Cobas e 601 which can measure numerous hormones and chemicals present in the blood serum sample. For this study, vitamin D (25[OH]D), T3, T4 and TSH are estimated and the values are noted. The machine works on the principle of

electrochemiluminescence (ECL) technology for immunoassay analysis. It combines Electrogenerated Chemiluminescence Immunoassay Technology (ECL) and magnetic particles to achieve highly sensitive analysis. The ECL labeled reagent or a sample reacts with the magnetic particles and forms an immunocomplex. The immunocomplex and the Ru2+ (tris-bipyridyl ruthenium metal cation) are attracted by the electrode by magnetism. For luminescence efficiency tris-bipyridyl ruthenium metal cation experiences an oxidation, electrochemical in nature and transitions to excited state to Ru3+. This state returns to the ground state releasing energy as light of a specified voltage. The amount of light emitted is proportional to the weight of the immunocomplex and thus the weight of the sample. It is therefore used for quantitative measurement [3].

For Serum 25(OH)D

This assay is intended for the quantitative determination of total 25-hydroxyvitamin D in human serum and plasma. This assay is to be used as an aid in the assessment of vitamin D sufficiency. The electrochemiluminescence binding assay is intended for use on Elecsys and Cobas e immunoassay analyzers. The Elecsys Vitamin D total II assay employs a vitamin D binding protein (VDBP) labeled with a ruthenium complex) as capture protein to bind 25-hydroxyvitamin D3 and 25-hydroxyvitamin D2. Cross-reactivity to 24,25-dihydroxyvitamin D is blocked by a specific monoclonal antibody [4].

For Serum T3

The immunoassay for determination of total triiodothyronine Immunoassay for the in vitro quantitative determination of total triiodothyronine in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and Cobas e immunoassay analyzer [5].

For Serum T4

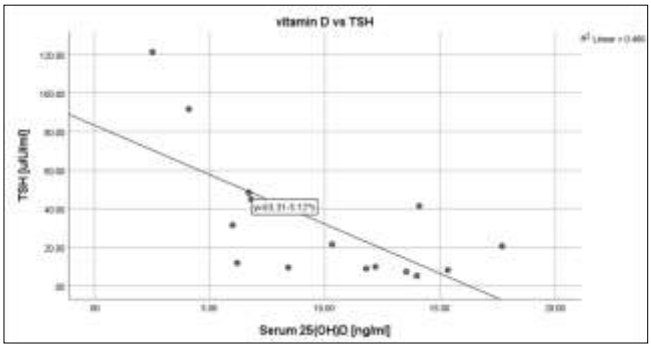
Immunoassay for the in vitro quantitative determination of thyroxine in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and Cobas e immunoassay analyzers. The Elecsys T4 assay employs a competitive test principle with an antibody specifically directed against T4. Endogenous T4, released by the action of 8-anilino-1-naphthalene sulfonic acid (ANS), competes with the added biotinylated T4-derivative for the binding sites on the antibodies labeled with the ruthenium complex [6].

For Serum TSH

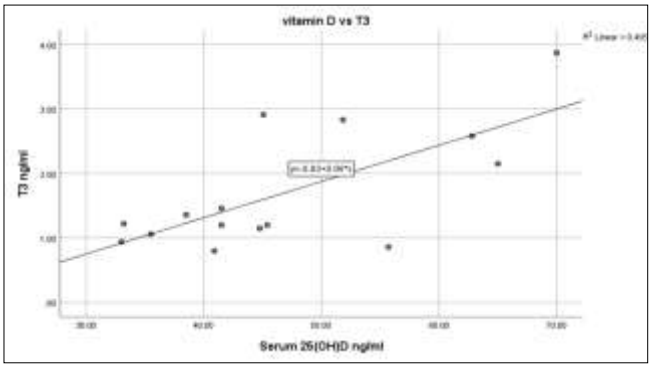
Immunoassay for the in vitro quantitative determination of thyrotropin in human serum and plasma. The electrochemiluminescence immunoassay "ECLIA" is intended for use on Elecsys and Cobas e immunoassay analyzers. The Elecsys TSH assay employs monoclonal antibodies specifically directed against human TSH. The antibodies labeled with ruthenium complex consist of a chimeric construct from human and mouse-specific components. As a result, interfering effects due to HAMA (human anti-mouse antibodies) are largely eliminated [7].

Results and Discussion

This study shows that the r value calculated in female and male cases between vitamin D as a non-dependent factor and TSH and T3 as a dependent factor. The study helps to find a significant correlation between vitamin D and TSH and T3. When this is compared to controls or healthy males and females it shows a positive moderate correlation between the two. This study is conducted between the ages 40 to 60 years. The analysis for results was carried out in SPSS for windows. T4 is not being considered a good value because it has unsatisfactory results.



Graph 1: The graph for female cases vitamin D vs TSH



Graph 2: Graph for male controls between vitamin D and T3.

Table 1: Table for mean of all values

Parameters: mean±SD	Cases	Controls
SEX	15 Males 50% 15 Females 50%	15 Males 50% 15 Females 50%
AGE (years)	F=51.2±6.90 M=50.4±5.85	F= 52.57± 6.9 M=48.46±6.74
Serum 25(OH)D	F= 9.98±4.52 M=15.60±7.74	F=48.32±10.25 M=46.98±11.65
Serum TSH	F=32.148±33.947 M=21.55±21.58	F=2.10±1.21 M=2.12±1.03
Serum T3	F=1.0043±0.182 M=1.09±0.19	F= 0.99± 0.182 M= 1.70±0.93
Serum T4	F=8.385±1.93 M=8.247±1.73	F= 9.062±1.934 M= 9.80±2.057

Table 2: Table for Pearson's correlation values

	T3		T4		TSH	
	Case	Control	Case	Control	Case	Control

Pearson correlation (r)	Male=-0.212 Female=0.567	Male= 0.703 Female R= 0.597	Male=0.0528 Female= -0.306	Male=-0.338 Female=0. 134	Male=-0.722 Female=-0.683	Male=-0.554 Female= -0.597
N	15	15	15	15	15	15

The results for this study was observed to be relevant towards the study. The mean values SD of all studied groups are in table (1) and the Pearson's correlation is studied in the table (2). There was not much significant statistical difference between groups regarding age and sex. Statistical analysis and results of serum 25(OH)D and T3, T4 and TSH levels in the groups for men and women of ages 40 to 60 are given in table (1) and (2). Since, for r value, closer the value is to 1, the more significant is the value. We observe that in men and women cases, vitamin D against TSH, the r value has slight difference (for women, r = 0.682 and for men, r = 0.722) and has a negative strong correlation meaning for every rise in vitamin D levels there is a significant decrease in the TSH levels. Whereas, for vitamin D as the independent variable with respect to T3, the female values are positively moderately correlated with each other, but the male cases show positive low correlation (r= 0.212). comparing this with the controls, male and female values show moderate correlation.

In controls vitamin D against TSH, male and female r values show moderate positive correlation and for the vitamin D and T3 graph, the value of r are in moderate correlation with each other.

T4 levels are not taken into consideration because of their uneven results and the fact that they show insignificant values**.**

[1] The Vitamin D Receptor in Thyroid Development and Function

Vitamin D is known to modulate thyroid neoplastic and autoimmune disease. The role of the vitamin D receptor (VDR) in normal thyroid development and function (thyrocytes and C cells) was investigated. In this research article, the thyroid phenotypes of VDR knockout mice and wild-type controls were differentiated and observed under a diet to overcome the effects caused due to hypocalcemia. Results showed that thyroid morphology unaffected in VDR knockout mice and expression of different parameters of thyrocyte function was comparable. Although, C cell physiology was lowered in the lack of the VDR, proceeding in raised thyroidal calcitonin expression, paralleled by raising serum calcitonin levels, but only in mice with normal calcium levels. In conclusion, the VDR is ample for normal thyrocyte function, but not for C cell function, where it mediates for the negative control of calcitonin by 1,25-dihydroxyvitamin D3. In human patients, basal serum calcitonin levels are not affected by vitamin D but requires more research.

[2] Relationship of Vitamin D Levels in Thyroid Malignancy

Antiproliferative, anti-apoptotic and pro-differentiating effects of vitamin D in several tumor types have been reported by several scientific research articles. The role of vitamin D in thyroid malignancy remains unknown. The study was conducted with 25-hydroxyvitamin D levels from 255 subjects receiving total (n=197) or completion (n=58) thyroidectomy for benign (n=190) and malignant (n=65) disease at the University of Arkansas for Medical Sciences between October 2005 and July 2013. No significant difference was observed between postoperative 25-hydroxyvitamin D levels in subjects with malignant pathology (median 25 ng/mL; range 21-37 ng/ml) versus benign pathology (median 27 ng/ml; range 19.5-35 ng/ml). In this study, there was no correlation between postoperative 25-hydroxyvitamin D status and pathological findings in subjects undergoing thyroidectomy.

[3] Thyroid Disease and Vitamin D Deficiency

Recent studies have shown vitamin D as an immunity-modulator low levels resulting in autoimmune diseases. 50 patients with AITD were compared with 42 non-autoimmune thyroid disease patients and 98 healthy subjects. The prevalence of vitamin D deficiency was observed to be significantly higher in patients with AITD compared to healthy individuals. Vitamin D deficiency significantly correlated with the presence of anti-thyroid antibodies and a trend towards an association with disturbed thyroid function was also observed. As an outcome, notably low levels of vitamin D were reported in patients with AITD, were related to the presence of anti-thyroid antibodies and disturbed thyroid function. While further studies are required, supplementation for vitamin D should be considered.

[5] Association of Vitamin D Deficiency with Hypothyroidism in Nepalese Population: A Pilot Study.

A broad array of non-skeletal diseases has been exhibited by vitamin D in recent years. A study for the relationship between vitamin D deficiencies in Hypothyroidism in a population of central Nepal. A total of 100 healthy individuals including 42 females and 58 males, where 100 hypothyroid patients including 20 males and 80 females were included in the study. Levels of Vitamin D, Thyroid function test (FT3, FT4, and TSH), Thyroperoxidase Antibody (Anti-TPO) was tested by electrochemiluminescence method and Calcium level was determined in accent 200 (PZ Cormay S.A, Poland), in Department of Immune serology and Biochemistry of United Reference Laboratory, Pokhara, Nepal. Statistical Analysis was done by Statistical Package for the Social Sciences (SPSS) Version 20.0. The mean age of the study subjects was 43 ±15 years (range 18-83) and 43 ±14(range 20-87) years for control group with no statistically significant difference, P= 0.933. The percentage of abnormal results comparison within study group for male and female was found to be statistically significant in only for TSH (80% and 80%). Correlations between Vitamin D and thyroid function test indicate that; for males there was a negative correlation of Vitamin D in all thyroid function tests, which was not statistically significant. For females the results indicate there was only a positive correlation between (Vit. D level and FT4) which was statistically significant (r =0.226, n = 80). In conclusion, the study indicates the Vitamin D deficiency is prevalent in hypothyroid patients, which suggests that they need routine testing and supplementation for the same.

Conclusion

- This study suggests that there may indeed be a close

correlation between vitamin D and hypothyroidism.

- Here we see that hypovitaminosis of vitamin D is in a negative significant correlation with hypothyroidism which suggests that depression in the levels of serum 25(OH)D, there is a rise in serum TSH levels.
- It is also seen that there is no significant difference seen between the male and the female correlations.
- The patients were mostly found between the ages 40 to 60 years.
- This suggests that screening for vitamin D is recommended for all hypothyroidism patients.

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