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Original Research Article

Effect vitamin D supplementation on Haematological parameters in hypothyroid subjects

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Abstract: ***Introduction:*** Malnourishment is one of the major public health challenges in India. And second largest disorder is the thyroid after diabetes.as thyroid hormone is necessary for normal erythropoiesis; anaemia often accompany thyroid disturbances. It is also observed that iron deficiency anaemia state negatively influences the thyroid hormone function. Vitamin D levels also associated with thyroid disorders and anaemia. So, the supplementation of vitamin D can influence the thyroid synthesis and improves the erythropoiesis. the relationship between anaemia and vitamin supplementation in thyroid disorders have to be addressed. Keeping this in view, we tried to study the vitamin D supplemented haematological parameters of inpatients with thyroid disorders in our hospital. ***Objectives***: Our study aims to estimate the relationship between vitamin D supplemented thyroid patients' haematological profile and non-supplemented vitamin D hypothyroid patients' haematological profile in comparison to controls. ***Methods:*** A cross-sectional study of 8 months period was conducted among 90 subjects attending the General Medicine department of Shadan Institute of Medical Sciences. Based on the inclusion and exclusion criteria, 90 subjects were divided into 3 groups, G-1(controls=30), G-2 (Vitamin D supplemented hypothyroid subjects=30), and G-3 (without vitamin D supplemented hypothyroid subjects=30). Healthy age and gender-matched euthyroid subjects were taken as controls and patients who were newly diagnosed as hypothyroid, (with increased serum TSH and or with decreased serum T3 or serum T4 levels) were taken as cases. Levels of serum vitamin D, & haematological parameters. were compared between groups. ***Results:*** 90 subjects participated in our study; the subjects were age matched and female predominance was observed. The haemoglobin levels were improved and found to be statistically significant, in vitamin D supplemented hypothyroid patients. Serum TSH, T3, and T4 levels were within the optimal range. There was not much difference in total WBC count but an increase in total RBC count. with a statistical significance was noted in the vitamin D supplemented hypothyroid patients. When vitamin D supplemented hypothyroid patients and without vitamin D supplemented hypothyroid patients were compared, total lymphocyte count decreased and improved total RBC count & haemoglobin concentration levels of vitamin D supplemented hypothyroid patients, a positive correlation was found, along with statistical significance, when tested by ANOVA. ***Conclusion:*** Hypothyroidism is found to be prevalent in females and is associated with mild anaemia. When vitamin D supplemented hypothyroid subjects and without vitamin D supplemented hypothyroid subjects were compared, there was a significant improvement in total RBC count & HB % levels supporting the advisability of vitamin D supplementation for hypothyroid patients.

Keywords: Anaemia, Haemoglobin, Hypothyroidism, Vitamin D.

INTRODUCTION

Thyroid disorders and anaemia are common disorders in the population, and their prevalence increases with age

(1–2). now a days decreased vitamin D levels also globally increasing. Hypothyroidism and anaemia can each cause nonspecific symptoms of ill health like fatigue Hypothyroidism are a clinical disorder due to deficiency of thyroid hormones, resulting in the reduction of metabolic processes (3). It affects the various systems of the body, particularly nervous, cardiovascular, pulmonary, reproductive, and renal organ systems (4). Anaemia is observed in all the age groups, especially in adolescent girls and women of child bearing age. There is an increase in the thyroid disorders in the country since the last decade. Thyroid hormone is indispensable for the normal erythropoiesis to occur in the bone marrow. Thyroid hormones stimulate the proliferation of erythrocyte precursors directly and also by increasing the production of erythropoietin. Hence, anaemia often accompanies thyroid disorders. It is also observed that iron deficiency anaemia state negatively influences the thyroid hormone function (5). Vitamin D plays a substantial role in lowering the occurrence of autoimmune diseases and maintenance of vitamin D levels is of much importance in case of deficiency of thyroid hormones (6-8). Thyroid hormone and vitamin D binds to identical receptors known as steroid hormone receptors. A gene in the Vitamin D receptor was revealed to predispose individuals to autoimmune thyroid diseases (9). Vitamin D facilitates its effect by binding to VDR, (Vitamin-D Receptor) which leads to the activation of VDR genes, but autoimmune thyroid diseases are associated with VDR gene polymorphism [10,11]. Hence, patients suffering from thyroid problems must try to understand vitamin D's importance.

MATERIALS AND METHODS

A cross-sectional study of 8 months period was conducted among 90 subjects attending the General Medicine department of Shadan Institute of Medical Sciences. The study comprised of subjects of either gender, aged between 15-45 years. All the subjects were instructed to fill informed consent form before enrolment in the study. An ethical committee permission letter was taken from the institution before the commencement of the study.

Group Allocation
90 subjects were divided into 3 groups
Group-1=Healthy controls (n=30)
Group-2=Vitamin D supplemented hypothyroid subjects (n=30)
Group-3=Without Vitamin D supplemented hypothyroid subjects (n=30)

Inclusion Criteria
Controls: Healthy age and gender-matched euthyroid subjects.
Cases: Newly diagnosed hypothyroid subjects (with increased serum TSH and or with decreased T3 or serum T4 levels).

Exclusion Criteria: Subjects are already on hypothyroid medication. Subjects suffering from any cardiovascular pulmonary, renal, neurological, or reproductive disorders.

Investigations Done: Thyroid profile: Done by Chemiluminescence Immunoassay (CLIA) [12].
Vitamin D: Done by Mini Vida's automated immunoassay analyzer [13].
Haematological parameters by automated analyser.

Five ml of blood sample was collected from all the subjects for estimation of biochemical parameters.
Serum was separated from collected blood and free T3, free T4, and TSH were assayed by using the Chemiluminescence Immunoassay (CLIA) method. Quantitative assay of vitamin D was done by using Mini Vida's automated immunoassay analyser and Haematological parameters autoanalyzer respectively.

Statistical Analysis: Executed by using the SPSS software. Data analysis was done by statistical tools, a) Descriptive analysis, b) student t-test, c) Chi-square test, d) Pearson's correlation and e) ANOVA.

RESULTS

90 subjects who participated in our study, were age-matched (mean age 33 years) and female predominance was observed (controls=69.2% and cases=55.3). Thyroid Stimulating Hormone (TSH): The mean value of thyroid stimulating hormone in control and Hypothyroidism are 2.84 ± 0.1 and 18.0 ± 2.3 (mIU/L [milli-international units per litter]) respectively. Compared to the control the Hypothyroidism group showed significant increase in thyroid stimulating hormone ($P < 0.001$). as well T3& T4 significantly decreases between cases to controls.

Table :1 comparison of thyroid profile (TSH, T3&T4) between controls & cases

Parameter	Group	Mean	SE	Analysis
TSH (mIU/L)	Control	2.84	0.1	t = -4.6 P < 0.01
	Hypo thyroid	18.0	2.3	
Free T_3 (pmol/L)	Control	1.19	0.0	t = -4.075 P < 0.001
	Hypo thyroid	3.8	0.4	
Free T_4	Control	5.5	0.2	t = -2.8 P < 0.0041
	Hypo thyroid	8.1	0.6	

Table :2 comparison of thyroid profile (TSH, T3&T4) between with and without vitamin-D supplementation hypothyroid subjects.

S.No	Parameter	Group	Mean	SE	Analysis
1	TSH Miu/ml	No Vit D	5.7	0.4	t = 0.8 P < 0.041
		Vit D Suppl	4.9	0.9	
2	fT Pg/ml 3	No Vit D	2.4	0.3	t = 2.8 P < 0.06
		Vit D Suppl	1.5	0.05	
3	fT ng/dl 4	No Vit D	6.68	0.3	t = 0.35 P < 0.071

Table :3 showing Thyroid stimulating hormone (TSH): The mean value of Thyroid stimulating hormone in no-vitamin D supplementation group and vitamin D supplemented group are 5.7 and 4.9 (Miu/mL), statistically p value is significant along with free Triiodothyronine (fT_3): The mean value of Triiodothyronine fT_3 in no-vitamin D supplementation group and vitamin D supplemented group are 2.4 and 1.5 (Pg/mL). Thyroxin (fT_4) The mean value of Thyroxin fT_4 in no-vitamin D supplementation group and vitamin D supplemented group are 6.68 and 6.4 (ng/dL).

Table :3 Comparison of Haematological profile controls & hypothyroid subjects.

S.No	Parameter	group	Mean	SE	Analysis
1	RBC mm^3	Control	4.04	0.03	t = 2.48 P < 0.01
		Hypo thyroid	3.8	0.06	
2	WBC mm^3	Control	6617	7.9	t = -1.2 P < 0.22
		Hypo thyroid	7209	7.2	
3	Haemoglobin g%	Control	11.8	0.1	t = 6.17 P < 0.001
		Hypo thyroid	10.1	0.1	
4	Neutrophil%	Control	60.8	1.5	t = -0.01 P < 0.901
		Hypo thyroid	60.8	0.8	
5	Lymphocyte%	Control	26.7	0.5	t =-2.5 P < 0.01
		Hypo thyroid	29.9	0.9	
6	Monocyte%	Control	4.7	0.1	t =1.4 P < 0.13
		Hypo thyroid	4.2	0.5	
7	Eosinophil%	Control	3.6	0.1	t = -0.3 P < 0.731
		Hypo thyroid	3.6	0.1	
8	Basophil%	Control	0.03	0.0	t = 0.0 P < 0.91
		Hypo thyroid	0.03	0.0	

RBC's: The mean value of RBCs in control and Hypothyroidism are 4.04 ± 0.03 and 3.8 ± 0.06 respectively. Compared to the control the Hypothyroidism group showed significant decrease in blood
RBC count. (P < 0.01). WBC's: The mean value of WBCs in control and Hypothyroidism are 6617 ± 7.9 and 7209 ± 7.2 respectively. Compared to the control the Hypothyroidism group showed significant increase in blood WBC count. (P < 0.22). Haemoglobin: The mean value of haemoglobin in control and Hypothyroidism are 11.8 ± 0.1 and 8.1 ± 0.1 respectively.

Table :4 Comparison of Haematological profile between with and without vitamin -D supplementation of Hypothyroid subjects.

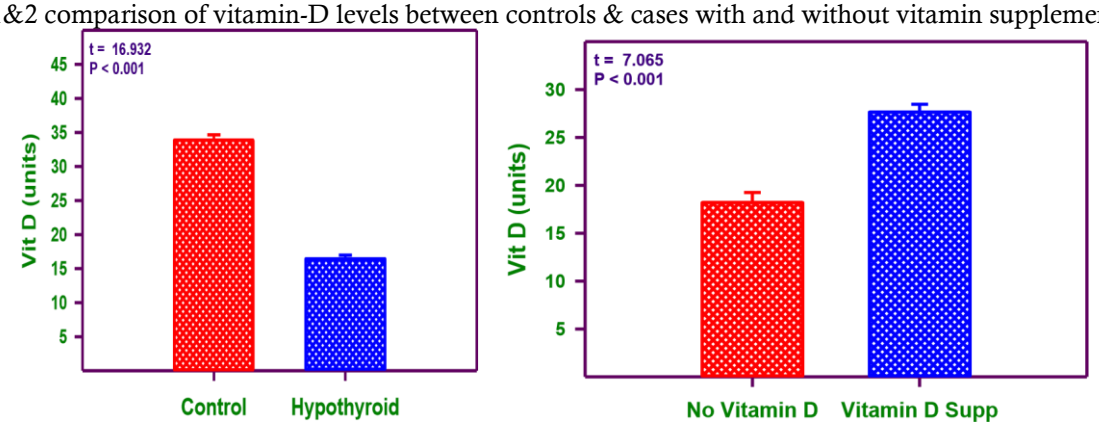
S.No.	Parameter	Groups	Mean	SE	Analysis
1	RBC mm^3	No Vit D	3.9	0.09	t = -2.01 P < 0.041
		Vit D Suppl	4.1	0.06	
2	WBC mm^3	No Vit D	7225	5.4	t = -2.16 P < 0.031
		Vit D Suppl	8061	5.5	
3	Hb g%	No Vit D	10.6	0.3	t = -3.93 P < 0.021
		Vit D Suppl	12.1	0.2	
4	Neutrophil%	No Vit D	60.7	0.9	t =0.7 P < 0.401
		Vit D Suppl	59.5	1.3	
5	Lymphocyte%	No Vit D	30.3	1.2	t = -1.1 P < 0.201
		Vit D Suppl	32.5	1.3	
6	monocyte%	No Vit D	3.9	0.15	t = -0.18 P < 0.801
		Vit D Suppl	4.0	0.14	
7	Eosinophil%	No Vit D	3.6	0.11	t = -0.66 P < 0.71
		Vit D Suppl	3.7	0.17	
8	basophil%	No Vit D	0.0	0.0	t = -2.0 P < 0.91
		Vit D Suppl	0.3	0.1	

Red Blood Cell Count The mean value of RBC count in no-vitamin D supplementation group and vitamin D supplemented group are 3.9 and 4.1 (million cells/Cumm), respectively. The mean value of WBC count in no-vitamin D supplementation group and vitamin D supplemented group are 7225 and 8061 (cells/Cumm), respectively. respectively. Value is significant (0.005).

Haemoglobin: The mean value of Haemoglobin in no-vitamin D supplementation group and vitamin D supplemented group are 10.6 and 12.1 (g/dL), respectively. Value is significant (0.005).

Neutrophils: The mean value of Neutrophils in no-vitamin D supplementation group and vitamin D supplemented group are 60.7 and 59.5. Lymphocyte: The mean value of Lymphocyte in no-vitamin D supplementation group and vitamin D supplemented group are 30.3 and 32.5 Monocyte: The mean value of Monocyte in no-vitamin D supplementation group and vitamin D supplemented group are 3.9 and 4.0, respectively. Eosinophil: The mean value of Eosinophil in no-vitamin D supplementation group and vitamin D supplemented group are 3.6 and 3.7 cells of differential WBC S are statistically not significant.

Graph :1&2 comparison of vitamin-D levels between controls & cases with and without vitamin supplementation



Serum Vitamin D: The mean value of Serum Vitamin D in control and Hypothyroidism are 33.820 ± 0.88 and 16.003 ± 0.61 (ng/mL) respectively. Compared to the control the Hypothyroidism group showed significant decrease in Serum Vitamin D (P < 0.001).

DISCUSSION

The whole purpose of our entire study was to analyse the haematological parameters before and after vitamin D supplemented hypothyroid patients. In our study, a total of 90 subjects participated. Out of 90 subjects, healthy age and gender-matched euthyroid subjects were taken as controls, and patients who were newly diagnosed as hypothyroid, (with increased serum TSH and or with decreased serum T3 or T4 levels) were taken as cases. Both the controls and cases were age-matched (mean age 33 years) and female predominance was observed (controls=69.2% and cases=55.3%). In a similar study reported by Morganti S, et al., a higher prevalence rate of hypothyroidism in women with advancing age was detected (14). The study by Unnikrishnan A, et al., on hypothyroid. patients, has revealed that the female gender has a significant association with hypothyroidism (15). In hypothyroidism, iron deficiency anaemia is usually attributed to malabsorption of iron and blood loss by menorrhagia. Thyroid hormones influence on haematopoiesis involves an increased production of erythropoietin or haematopoietic factors by non-erythroid cells. Banday TH et al found that about one fifth of the patients with hypothyroidism were iron deficient. Iron is an important component of many enzymes in the thyroid hormone synthesis including Thyroid Per-Oxidise (TPO). Low ferritin levels have been observed in patients with hypothyroidism (16). hypothyroidism and iron deficiency states are intensely interrelated. iron deficiency is very common in general population in India and prevalence of hypothyroidism has seen a steep rise over the past few years in the country.

In our study, a prominent difference was noted in serum TSH, T3, and T4 levels of vitamin D supplemented hypothyroid subjects compared to those without vitamin D supplemented hypothyroid subjects. Serum TSH, T3, andT4 levels were within the optimal range, in controls and cases study groups. A similar result was observed by Simsek Y, et al., improved Haemoglobin levels and RBC count has been noted in vitamin-D supplemented hypothyroid subjects.

CONCLUSION

The results in our study show that hypothyroidism is found to be prevalent in females and is associated with mild anaemia. When vitamin D supplemented hypothyroid subjects and without vitamin D supplemented hypothyroid subjects were compared, there was a significant improvement has seen in haematological profile advisability of vitamin D supplementation for hypothyroid patients.

Maintenance of vitamin D levels is recommended for all patients suffering from hypothyroidism.

DECLARATIONS Conflicts of Interest

The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

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