RESEARCH
O&G Forum 2024; 34- 3s: 2233-2237

A STUDY ON SERUM VITAMIN D AND FERRITIN LEVELS IN FEMALES DIAGNOSED WITH HYPOTHYROIDISM

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Abstract
Introduction: Thyroid hormones are synthesized and released by the thyroid gland. Vitamin D and thyroid hormones both act through similar nuclear receptors and are expected to affect each other's action. Vitamin D receptors are located in thyroid tissue also and by binding with its receptor, vitamin D may play a role in maintaining a normal thyroid function. Acting as an immune modulator, vitamin D reduces activation of the acquired immune system and its deficiency is suggested to increase the risk of autoimmune diseases including thyroid diseases. Synthesis of thyroid hormones requires an iron containing enzyme, thyroid peroxidase (TPO). Iron inadequacy can affect the proper functioning of this enzyme that further affect the thyroid hormone production. Serum ferritin is an iron storage protein and an index of iron store. This study was conducted to assess association of vitamin D and serum ferritin with thyroid stimulating hormone (TSH) levels in female hypothyroid patients and to compare the levels of vitamin D and ferritin with healthy controls.

Materials and Methods: The present study was hospital based cross sectional study carried out in the department of Biochemistry in collaboration with department of Medicine at SGT Medical college, Gurugram, Haryana. Total 160 female subjects aged between 20-50 years comprising of 80 hypothyroid female subjects as cases and 80 age matched healthy subjects as controls were included in the study. Fasting blood samples were collected from all subjects enrolled in the study and evaluated for thyroid profile (FT3, FT4 and TSH), Vitamin D and ferritin by chemiluminescence immunoassay (CLIA) on MAGLUMI 1000 fully autoanalyser. Results were subjected to statistical evaluation and presented as mean ± SD. P value ≤ 0.05 was considered as statistically significant.

Results: The mean value of TSH of hypothyroid patients was found higher (15.74 ± 13.96 µIU/ml) than that of control group (3.01 ± 0.78 µIU/ml) whereas the mean value of FT3 (2.43 ± 0.55 pg/ml), FT_4 (1.14 ± 0.19 ng/dl), vitamin D (16.84 ± 8.2 ng/ml) and ferritin (36.56 ± 27.82 ng/ml) were found to be reduced in patients with hypothyroidism compared to normal subjects. All the parameters were statistically significant with p value (<0.001).
Conclusion: The present study showed that hypothyroid subjects had significantly lower serum vitamin D and ferritin concentration than healthy controls. There was a significant negative correlation between vitamin D and TSH. Similar negative correlation was found between ferritin and TSH. Hypothyroidism is associated with low serum vitamin D and ferritin levels and their measurement could be beneficial in monitoring of these patients. Along with it regular screening of vitamin D and ferritin levels could be helpful in maintaining their proper levels which will be beneficial in reducing the complications occurring as a consequence of the deficiency.
Keywords: Hypothyroidism, Thyroid stimulating hormone (TSH), vitamin D, Ferritin.

INTRODUCTION

Thyroid disorders are extremely common worldwide. According to the studies, approximately 42 million people in India suffer with thyroid diseases. The prevalence of sub-clinical hypothyroidism and overt hypothyroidism is 9.4% and 3.9% respectively.[1] Also it is more common among women. Thyroid gland synthesizes thyroid hormones and these hormones have many effects on metabolic processes and are essential for cell growth, differentiation and development. Hypothyroidism is an endocrine disorder in which sufficient amount of thyroid hormones are not synthesized by the thyroid gland and the person has slower metabolism.

Vitamin D deficiency is increasing worldwide and is the most common untreated nutritional deficiency.[2] Over billions of people are vitamin D insufficient (serum 25(OH) D of 10-29 ng/ml) or vitamin D deficient (serum 25(OH)D less than 10ng/ml[3,4] which associates with the risk of chronic illnesses including cardiovascular, infectious diseases and autoimmune diseases.[5] Vitamin D is ingested in the diet but mostly generated through the skin and affects the expression of several genes.[6] Vitamin D plays an important role in avoiding the onset of many infectious, inflammatory and autoimmune illnesses.[7] Vitamin D insufficiency is linked to a number of autoimmune illnesses.[8] Vitamin D receptors can be present in a variety of organs including thyroid gland. Vitamin D and thyroid hormones both act through similar nuclear receptors and are expected to affect each other's action as they have similar response elements on gene. A reduced level of vitamin D is likely to aggravate the systemic abnormalities associated with hypothyroidism.[5,9] Studies assessing the association between vitamin D levels and hypothyroidism have reported conflicting results. Few symptoms of vitamin D deficiency overlap with that of the hypothyroidism.[10]

Minerals and trace elements such as iodine, iron, selenium, and zinc are required for the thyroid gland to function properly. Iron is stored in the form of ferritin. Total body iron can be determined by markers such as serum ferritin, total iron binding capacity and serum iron etc. Ferritin is considered as the best single reliable indicator of total body iron.[11] Thyroid peroxidase (TPO) plays a significant role in the biosynthesis of thyroid hormones by organification. It is a membrane-bound glycosylated hemoprotein and the initial two steps of biosynthesis of thyroid hormones are catalysed by this enzyme. Therefore iron levels are associated with the levels of thyroid hormone in the body. Deficiency of iron has been reported to diminish the body's ability to synthesize thyroid hormone.[12]

Iron as a cofactor for many enzymes and as a component of hemoproteins, play a very important role in the body. Contrary to this chemical properties of iron can cause damage to the biological systems. Therefore, to maintain the levels of iron within a normal range is very important. As decrease in iron level may cause anemia whereas high levels causes damage to the tissues.[13,14] According to some studies, hypothyroidism causes immunosupression that may lead to increased oxidative stress and a reduction in the level of iron storage markers had been observed in hypothyroid subjects.[15]

Therefore in the present study we aimed to determine the serum vitamin D and ferritin levels in female hypothyroid patients and to assess the correlation between vitamin D, ferritin and thyroid stimulating hormone.

MATERIALS AND METHODS

The present study was a hospital based cross sectional study carried out in the department of Biochemistry in collaboration with department of Medicine at SGT Medical college, Gurugram, Haryana. Total 160 female subjects aged between 20-50 years comprising of 80 hypothyroid female subjects as cases and 80 age matched healthy subjects as controls were included in the study. The study was conducted after seeking approval from the institutional ethical committee and written informed consent were obtained from all participants. Under aseptic conditions 7 ml of venous blood samples were withdrawn from antecubital vein after 12-14 hours of overnight fasting condition from all the subjects and controls. Serum was separated by centrifugation at 3000 rpm for 15 minutes. Serum was taken for FT3, FT4, thyroid-stimulating hormone (TSH) levels, vitamin D and ferritin estimation. All parameters were estimated using chemiluminescence immunoassay (CLIA) on MAGLUMI 1000 auto analyzer.

Results obtained were presented as mean ± SD and subjected to statistical evaluation. The correlation of TSH with vitamin D and ferritin were studied by Pearson's correlation coefficient analysis. P value ≤ 0.05 was considered as statistically significant.

Inclusion criteria:

Cases were recruited from females in the age group of 20-50 years attending Medicine OPD of SGT Hospital and diagnosed with Hypothyroidism based on clinical examination and Laboratory findings.(TSH >5mIU/L and/or decreased levels of FT3 and FT4).[16]

Exclusion criteria:

Alcoholics and/ or chronic smokers, patients taking vitamin D supplements, pregnant and lactating women were excluded from the study.

RESULTS

Biochemical parameters including serum FT3, FT4, TSH, vitamin D and ferritin in hypothyroid female patients and healthy controls were compared in the present study. Analysis of statistical data was performed using the SPSS software (Statistical Package for the social Sciences, version 20.0). Mean and Standard Deviation of continuous variables were calculated. Data were analyzed using independent student 't' test for their level of significance. Correlation between variables was carried out using Pearson's coefficient of correlation. In results, p value <0.05 was considered statistically significant.

Table 1 shows the demographic details of the study subjects. The mean age ± SD among cases was 34.01 ± 8.28 years and among the controls was 32.59 ± 7.76 years. There was no significant difference with respect to age distribution in cases and controls (p=0.263, Table 1).

Table 1 also shows the comparison of the mean levels of FT3, FT4, TSH, Vitamin D and Ferritin between the cases and controls. In present study we found that there was a statistically significant difference in the thyroid profile between cases and controls. It was observed that the mean TSH of hypothyroid patients and controls was 15.75 ± 13.96 μIU/ml and 3.02 ± 0.78 μIU/ml respectively. The comparison of serum TSH levels between two groups were statistically significant with P value (<0.001). The mean FT3 of patients and controls was 2.43 ± 0.55 ng/ml and 2.78 ± 0.52 ng/ml respectively. The comparison of FT3 between two study groups was found to be statistically significant with P value <0.001. Similarly the mean FT4 levels in patients and controls was 1.14 ± 0.13 ng/ml and 1.35 ± 0.19 ng/ml respectively. Statistically significant difference with P value (<0.001) was observed. In current study, it was found that mean value serum vitamin D (16.84 ± 8.2 vs 30.04 ± 7.33 ng/ml) and ferritin (36.57 ± 27.82 vs 65.82 ± 37.37 ng/ml) was reduced in patients with hypothyroidism compared to normal subjects. The results were statistically significant with p value (<0.001).

Table 1. Comparison of parameters between patients with hypothyroidism and control group

Parameters	Cases Mean ± SD	Controls Mean ± SD	P value
Age	34.01 ± 8.28	32.59 ± 7.76	NS
TSH (µIU/ml)	15.75 ± 13.96	3.02 ± 0.78	<0.001
FT3 (pg/ml)	2.43 ± 0.55	2.78 ± 0.52	<0.001
FT4 (ng/dl)	1.14 ± 0.13	1.35 ± 0.2	<0.001
Vitamin D (ng/ml)	16.84 ± 8.2	30.04 ± 7.33	<0.001
Ferritin (ng/ml)	36.57 ± 27.82	65.82 ± 37.37	<0.001

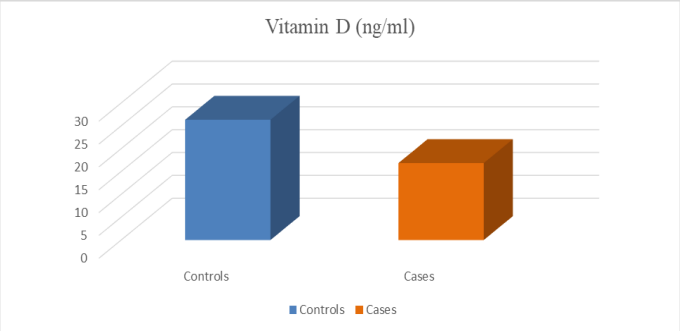

Figure 1. Comparison of Vitamin D between patients with hypothyroidism and controls showing significant decrease in Vitamin D (p<0.001) in patients

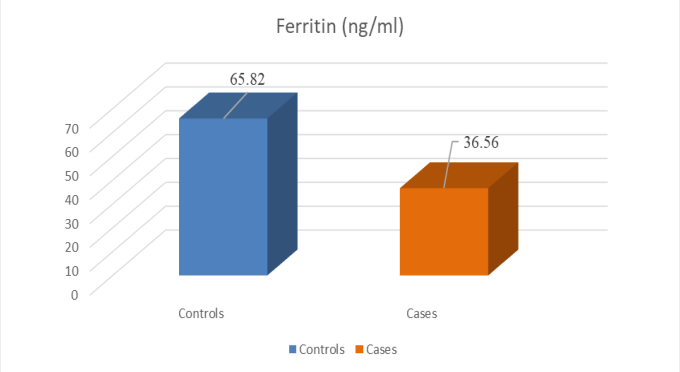

Figure 2. Comparison of Ferritin between patients with hypothyroidism and controls showing significant decrease in Ferritin (p<0.001) in patients

Correlation analysis :
Significant correlations were found between serum thyroid stimulating hormone with serum vitamin D and serum ferritin. Serum vitamin D showed significant negative correlation with serum thyroid stimulating hormone (r = -0.667, p < 0.001) (Table 2, Figure 3). Similarly, a significant negative correlation was found between serum ferritin and serum TSH (r = -0.471, p = <0.001) (Table 2 , Figure 4).

Table 2. Pearson's correlation coeficients of TSH with Vitamin D and Ferritin

Parameters	CorrelationCoffecient (r Value)	Significance (p Value)
Vitamin D	-0.667	< 0.001
Ferritin	-0.471	< 0.001

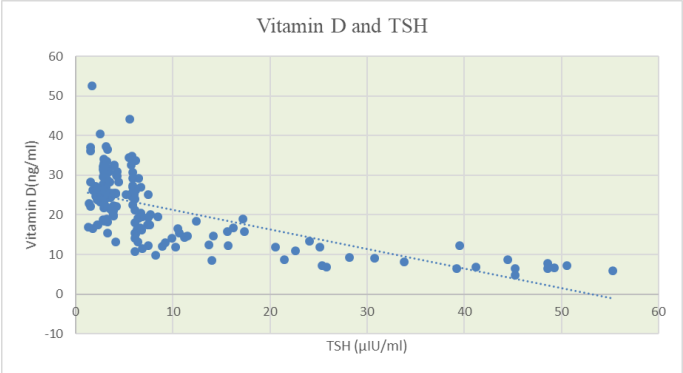

Figure 3. Correlation scatter plot graph between serum vitamin D and serum thyroid stimulating hormone among hypothyroid female subjects

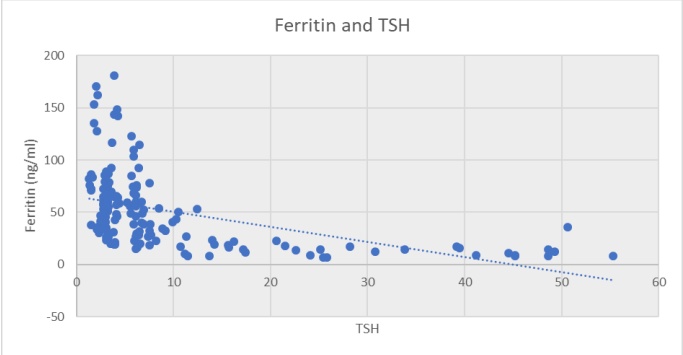

Figure 4. Correlation scatter plot graph between serum ferritin and serum thyroid stimulating hormone among hypothyroid female subjects

DISCUSSION

Vitamin D commences its action by binding to vitamin D receptor (VDR) which further activates the VDR-Responsive gene.[17] Main function of Vitamin D is said to be regulation of bone and calcium metabolism. In addition, Vitamin D affects the functioning of many body cells which possess VDRs. Vitamin D also binds with its receptor located in thyroid gland and may play an important role in maintaining the normal function of thyroid gland. Along with maintaining bone health and mineral metabolism, vitamin D plays various other important roles in the body such as controlling infection and inflammation. Vitamin D shows effects on cells of the immune system including antigen presenting cells, such as macrophages, dendritic cells, T and B cells which express vitamin D receptors. Acting as an immune modulator, vitamin D reduces activation of the acquired immune system and its deficiency is suggested to increase the risk of autoimmune diseases such as type 1 diabetes, rheumatoid arthritis, multiple sclerosis, Crohn's disease and autoimmune thyroid disease.[18-20] Research has found association of Vitamin D deficiency to autoimmune thyroid disorders such as Hashimoto's thyroiditis and Grave's disease. Also impaired vitamin D signaling has been related to thyroid cancer.[21]

In our present study we observed that the hypothyroid female patients had significantly lower levels of serum Vitamin D as compared to healthy controls (p<0.001). On Pearson's correlation analysis, we found a negative correlation between Vitamin D and thyroid stimulating hormone (TSH) levels (r =-0.667, p<0.001). Similar results have been reported by Esmat et al, Bizzaro and Shoenfeld, they reported association of vitamin D status with thyroid diseases.[22,23] They observed a strong correlation of vitamin D deficiency with increased incidence of autoimmune thyroiditis. Similarly, Shilpa et al in

their study on Indian population observed a relationship between Vitamin D and hypothyroidism that was statistically significant.[24] In a study conducted on hypothyroid patients with vitamin D deficiency, an improvement in thyroid function was observed after supplementation of vitamin D deficiency indicates a role of vitamin D in thyroid function.[25]

Tahir et al in their study reported that deficiency of serum vitamin D was associated with degree and severity of hypothyroidism and that was statistically significant.[26] Byron Richards et al conducted a study and have suggested that low thyroid activity in turn lead to deficiency of vitamin D.[27] Recently many studies have highlighted the role of Vitamin D as a potential immune modulator and have explained its influence on pathogenesis of autoimmune disease.[28,29] Shin et al suggested that vitamin D levels were an independent factor which affects the positivity of TPO antibody.[30]

Changes in serum ferritin levels have also been seen in subjects with thyroid diseases, indicating an association between serum ferritin concentration and thyroid functions. Present study focused to find correlation between serum TSH and ferritin levels in the female suffering with hypothyroidism. In current study, it was found that mean value of serum ferritin concentration in healthy controls was higher (65.82 ± 37.37ng/ml) as compared to the mean value of serum ferritin among hypothyroid subjects (36.56 ± 27.82 ng/ml) and it was found to be statistically significant with p value (<0.001). Our results are similar with the study conducted by Sachdeva A et al who concluded that serum ferritin levels were decreased in patients with hypothyroidism as compared to healthy individuals. Also reported that hypothyroidism is associated with low serum ferritin levels.[31] Similar results were observed by Sahana KR and Kruthi BN in their study.[16] In our study we found a negative correlation between TSH and ferritin levels in serum of female hypothyroid patients which was statistically significant ($r=-0.471$, $p< 0.001$).

Thyroid hormones have a significant impact on oxidative stress. Studies done by G Lenaz et.al suggested an increase in the production of Reactive Oxygen Species (ROS) in hypothyroidism which may result in oxidative stress.[32] Therefore increase in oxidative stress has been reported in hypothyroidism.

When body starts losing iron, the body can maintain its iron levels by drawing from its stores until they are all used up. Further the iron levels can no longer be maintained in their normal levels and will start to fall. By sequestering of iron, ferritin plays a role of protective antioxidant. When TSH concentration increases in the condition of hypothyroidism, the levels of antioxidants including ferritin decreases.[33] This suggests an association between TSH and ferritin. In addition to this significant alteration in serum ferritin levels in hypothyroid patients could be a reflection of disturbed activities of iron-dependent enzymes such as thyroid peroxidase, which impairs thyroid hormone metabolism but the exact mechanism by which thyroid hormone alters ferritin concentration is not well known. Further studies should be done to get a better perspective.

CONCLUSION

Our study intended to determine whether there was a link between vitamin D status, ferritin and hypothyroidism. According to our observations the majority of hypothyroid patients were vitamin D deficient. Further, there was a significant inverse relationship between vitamin D and TSH. In present study a decrease in serum ferritin levels was also observed in female patients suffering from hypothyroidism when compared to normal controls. According to the results we may conclude that vitamin D and ferritin is associated to hypothyroidism and their measurement could be beneficial in the monitoring of the patients. Estimation of these parameters may provide useful information in the prognosis of the disease. Along with it regular screening of vitamin D and ferritin levels could be helpful in maintaining their proper levels which will be beneficial in reducing the complications occurring as a consequence of the deficiency.

Source of Funding:
None.

Conflict of Interest:
The authors declare no conflict of interest.

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