Novel evidence of relationship between serum vitamin B_{12} and folate with total vitamin D in subclinical hypothyroidism

Amit Ranjan, Sudha K.*, Nandini M., Durgarao Y.

*Associate Professor, Department of Biochemistry, Kasturba Medical College, Mangalore, Manipal Academy of Higher Education, Manipal, Karnataka, India

(Received: May 2020 Revised: July 2020 Accepted: August 2020)

Corresponding author: **Sudha K.** Email: sudha.k@manipal.edu

ABSTRACT

Introduction and Aim: Subclinical hypothyroidism is an early form of thyroid dysfunction, which may develop into full-blown hypothyroidism in absence of proper dietary and medical intervention. Subclinical hypothyroidism (SCH) can induce metabolic derangements and increase risk of hypertension and cardiovascular disease. Hyperhomocysteinemia, a risk factor of CVD, is the most prevalent metabolic derangement in subclinical hypothyroidism. Thyroxine and vitamin D modulate the expression of genes and regulate the activity of methyl transferase needed for conversion of homocysteine to methionine, which requires folate and cobalamin as cofactors. The aim of the study is to establish the interrelationship between the three vitamins in SCH.

Materials and Methods: Study population included 100 freshly diagnosed subclinical hypothyroid patients and 50 age and sex matched euthyroid adults. Serum TSH, T_3, T_4, total vitamin D, vitamin B_{12} and folate were estimated by electrochemiluminescence Immunoassay (ECLIA) using Cobas 6000 autoanalyzer.

Results: Serum TSH was significantly high in SCH patients compared to euthyroid subjects. Serum total vitamin D, vitamin B_{12} and folate decreased significantly in SCH patients compared to the controls with p values being 0.001, 0.05 and 0.01 respectively. There was a significant negative correlation between vitamin D and TSH ($r = -0.38$ $p=0.03$). Further, correlation between vitamin D with cobalamin ($r=0.46$, $p=0.009$) and with folate ($r=0.42$, $p=0.01$) were also statistically significant.

Conclusion: Based on the findings, it is imperative to link vitamin deficiencies with thyroid dysfunction and vitamin supplementation in SCH patients may prevent the progression to hypothyroidism.

Keywords: Subclinical hypothyroidism; cobalamin; folate; vitamin D; TSH.

INTRODUCTION

Subclinical hypothyroidism (SCH) is an early, mild form of thyroid dysfunction without obvious symptoms which is detected during routine thyroid function testing with a prevalence of 3-8% (1). If ignored, without proper intervention it can develop into full blown hypothyroidism within few years. In this endocrine disorder, serum TSH will be above the upper limit of reference range while T_3 and T_4 within the normal range. Micronutrients like minerals and vitamins play a vital role in maintenance of normal thyroid function and few publications have addressed their role in prevention and treatment of diseases. Hypothyroidism is often associated with vitamin D deficiency, while prevalence of folate and vitamin B_{12} deficiency in hypothyroidism is about 40% (2). Since the evidence linking vitamin deficiencies and thyroid dysfunction is growing, we aimed to evaluate these vitamins in SCH, an early stage of thyroid disorder. SCH can induce metabolic derangements and increase risk of hypertension and cardiovascular disease Folate deficiency may lead to elevation in homocysteine (Hcy), a risk factor of atherosclerosis. Thyroxine influences homocysteine metabolism by regulation of methyl transferase activity, while vitamin D modulates the expression of the genes involved. Lower vitamin D and elevated Hcy are both considered as potential factors for premature cardiovascular disease (3). Majority of studies have been conducted on relationships between vitamin D and B vitamins in CVD, though thyroid dysfunction is also a risk factor of CVD, no study has been done to examine the relationship between these vitamins in thyroid disorders. Currently, there is no published data proposing vitamin D as a link between SCH and the two water soluble vitamins, folate and cobalamin. Hence, the aim of the study is to establish the interrelationship between the three vitamins in SCH.

MATERIALS AND METHODS

Study population included 100 freshly diagnosed asymptomatic subclinical hypothyroid patients aged between 30 to 50 years and 50 age and sex matched euthyroid adults. Patients with serum TSH level more than 4.2µIU/ ml only were included in SCH group. Smokers, patients with hypertension, cardiac, hepatic and renal disorders were excluded from the study. Subjects on vitamin supplements and on medications affecting thyroid were also excluded from the study. This study was approved by institutional ethics committee and informed consent was collected from all the subjects. Fasting blood samples were collected in plain vacuum tubes and serum was used for

biochemical analysis. Vitamins and hormones were determined by electrochemiluminescence Immunoassay. TSH, T_3, T_4 were measured by using sandwich immunoassay technique, total vitamin D, vitamin B_{12} and folate were estimated by competitive immunoassay in Cobas 6000 autoanalyzer (4-6). SPSS software version 20 was used for statistical analysis. Data was analyzed by Mann Whitney test and correlation between vitamin D and other variables were tested by Pearson's coefficient. p value less than 0.05 was considered significant.

RESULTS

Serum TSH was significantly increased in SCH group compared to euthyroid subjects, however, T_3 and T_4 remained in the normal range in these patients. Serum total vitamin D, vitamin B_{12} and folate decreased significantly in SCH patients compared to the controls with p values being 0.001, 0.05 and 0.01 respectively (Table 1). Water-soluble vitamins showed positive correlation with serum T_3, T_4 and TSH (Table 2). Furthermore, correlation between vitamin D and vitamin B_{12} (r=0.46, p=0.009), vitamin D and folate (r = 0.42, p = 0.01) were also statistically significant. Further, correlation between vitamin D and TSH was statistically significant (Table 3).

Table 1: Comparison of thyroid profile and vitamins between SCH and euthyroid adults (mean ± SD).

	SCH (n = 100)	Normal (n=50)	p value
T_3 (ng/ml)	1.22 ± 0.3	1.12 ± 0.2	NS
T_4 (µg/dl)	7.68 ± 1.54	7.86 ± 1.42	NS
TSH (µIU/ml)	6.71 ± 1.46	2.03 ± 0.89	0.001
Vitamin B_{12} (ng/ml)	277.2 ± 37.89	314.85 ± 41.10	0.045
Folate (ng/ml)	5.77 ± 4.03	9.94 ± 2.52	0.004
Vitamin D (ng/ml)	18.69 ± 10.8	31.25 ± 9.09	0.001

n = number of patients; NS = not significant

Table 2: Correlation of vitamin B_{12} and folate with thyroid profile in SCH patients.

	Vitamin B_{12}		Folate	
	r	p	r	p
T_3	0.01	0.94	0.09	0.61
T_4	0.05	0.77	0.06	0.73
TSH	0.2	0.27	0.13	0.47

Table 3: Correlation of vitamin D with thyroid profile and other vitamins in SCH patients.

	r	p
T_3	0.23	0.19
T_4	0.27	0.13
TSH	- 0.38	0.03*
Vitamin B_{12}	0.46	0.009*
Folate	0.42	0.01*

*significant

DISCUSSION

Vitamins play a significant role in maintenance of thyroid function and may act as one of the key factors in prevention of diseases. There are growing evidence to show the association of hypovitaminosis and hypothyroidism. However, whether decrease in serum vitamin levels is a consequence or a cause of thyroid dysfunction is still unclear. Thyroid hormones are considered as catabolic hormones as they regulate various metabolic reactions and processes (7). Moreover, thyroid dysfunction is also a key risk factor for development of CVD. Latest concept that is emerging suggests that high TSH and low thyroid hormones may lead to subclinical atherosclerosis (8). Previous studies have demonstrated that hyperhomocysteinemia is an independent risk factor for atherosclerosis. Hyperhomocysteinemia has been reported both in subclinical hypothyroidism and hypothyroidism (9). Decreased activity of homocysteine methyl transferase was established in thyroidectomized rats, which explains elevated levels of Hcy in hypothyroidism (10). Thyroxine also influences reduction of glomerular filtration rate of Hcy (11). Further, methylation of Hcy requires two water-soluble vitamins viz., folate and vitamin B_{12} as coenzymes. Results of the present study demonstrates marked reduction in serum vitamin B_{12} and folate in subclinical hypothyroidism, which is in agreement with an earlier study done on pregnant women with SCH suggesting increased risk of development of CVD in these patients (12). Some studies showed that folate treatment decreased Hcy and levothyroxine replacement therapy in patients with hypothyroidism reduced the plasma levels of Hcy (13). Barbe *et al.,* (14) reported low serum vitamin B_{12} and folate in hypothyroidism, with a positive correlation between TSH and folate which is in accordance with our results on SCH. On the contrary, serum folate was higher, and cobalamin was lower in hypothyroid patients in another study (15). Gyftaki *et al.,* (16) observed lower folate and higher B_{12} in hyperthyroid patients. Vitamin D exerts a direct influence on hypothalamus pituitary thyroid axis (17) and deficiency was strongly associated with higher TSH levels even in euthyroid subjects (18). A similar observation was seen in our study where vitamin D deficiency was prevalent in SCH patients (4). Hypothyroid patients with vitamin D supplementation for three months improved serum TSH without any alteration in thyroid hormones. Thus, vitamin D may modulate TSH secretion by binding on to the receptors on the pituitary gland

(17). Chinese patients with hypertension and CVD showed highest level of Hcy in patients with lowest level of vitamin D. Further, there was a significant negative correlation between TSH and vitamin D in the patients with hypertension and CVD (3). Vitamin D is thought to modulate the expression of genes involved in Hcy metabolism (19). Mohammed et al (20) hypothesized that vitamin D may have an impact on Hcy level independent of folate or cobalamin status. Coexistence of SCH and vitamin D deficiency is found to deteriorate the diastolic function of heart (21). Vitamin D deficiency and vitamin D receptor gene polymorphism has been associated with autoimmune thyroid disorders (22) confirming the immunomodulatory effect of vitamin D in thyroid dysfunction. Vitamin B_{12} deficiency was also prevalent in autoimmune thyroid disorders (23). Our results indicate a significant positive correlation between vitamin D and the water-soluble vitamins viz., cobalamin and folate in SCH. We therefore postulate that low serum vitamin D may be an unappreciated link between high TSH and low water-soluble vitamins in SCH.

Findings of the study suggest that serum vitamin status in SCH mimics that of hypothyroidism. Dietary vitamin supplementation in SCH patients may prevent subclinical atherosclerosis, cardiovascular complications and aid in designing better therapeutic interventional modes to improve the quality of life and prevent the progression to hypothyroidism.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

1. George, R. W., Whit, C. R. Subclinical thyroid disease. Am Fam Physician. 2005; 72(8): 1517-1524.
2. Aryn, B. C., Roman, P. Prevalence of vitamin B12 deficiency among patients with thyroid dysfunction. Asia Pac J Clin Nutr. 2016; 25(2): 221-226.
3. Mao, X., Xing, X., Xu, R., Gong, Q., He, Y., Li, S., *et al.*, Folic Acid and vitamins D and B12 correlate with homocysteine in Chinese patients with type-2 diabetes mellitus, hypertension, or cardiovascular disease. Medicine(Baltimore). 2016; 95(6): e2652. doi: 10.1097/MD.0000000000002652
4. Sudha, K., Anupama, H., Poornima, A. M., Reshma, K. Prevalence of vitamin D deficiency and its relationship with subclinical hypothyroidism. Int J Pharm Bio Sci. 2013; 4(4): B 1380-1384.
5. Wang, Y. P., Lin, H. P., Chen, H. M., Kuo, Y. S., Lang, M. J., Sun, A. Hemoglobin, iron, and vitamin B12 deficiencies and high blood homocysteine levels in patients with anti-thyroid autoantibodies. J Formos Med Assoc. 2014; 113(3): 155-160.
6. Komaromy-Hiller, G., Nuttall, K. L., Ashwood, E. R. Effect of storage on serum vitamin B12 and folate stability. Ann Clin Lab Sci. 1997; 27: 249-253.
7. Ernst, A., Lien, S., Hustad, B. G., Nedrebø, O. N., Ureland, P. M. Total plasma homocysteine in hypo- and hyperthyroidism: covariations and causality The Journal of Clinical Endocrinology & Metabolism. 2001; 86(4): 1835-1846.
8. Eric, B., Philippe, G., Rita, C., Turpin, G. Low Free-thyroxine levels are a risk factor for subclinical atherosclerosis in euthyroid hyperlipidemic patients. Journal of Cardiovascular Risk. 1999; 6: 327-331.
9. Bamashmoos, S. A., Al-Nuzaily, M. A., Al-Meeri, A. M., Ali, F. H. Relationship between total homocysteine, total cholesterol and creatinine levels in overt hypothyroid patients. Springerplus. 2013; 30: 423. doi: 10.1186/2193-1801-2-423
10. Ayav, A., Alberto, J. M., Barbe, F., Brunaud, L., Gerard, P., Merten, M., *et al.*, Defective remethylation of homocysteine is related to decreased synthesis of coenzymes B12 in thyroidectomized rats. Amino Acids. 2005, 28: 37-43.
11. Ning, Y., Zhi, Y., Li, M., Jia, L., Xia, G., Hui, F., *et al.*, Novel clinical evidence of an association between homocysteine and insulin resistance in patients with hypothyroidism or subclinical hypothyroidism. PLoS ONE 2015; 0(5): e0125922. doi: 10.1371/journal.pone.0125922
12. Srilatha, B., Aparna, B. V., Rajagopalan, V., Sailesh, S. K. Association of vitamin B12 and folic acid with thyroid hormones in healthy pregnant women and pregnant women with subclinical hypothyroidism. Journal of Chemical and Pharmaceutical Sciences. 2017; 10(1): 297-303.
13. Deyneli, O., Akpınar, I. N., Meriçliler, O. S., Gözü, H., Yıldız, M. E., Akalın, N. S. Effects of levothyroxine treatment on insulin sensitivity, endothelial function and risk factors of atherosclerosis in hypothyroid women. Ann Endocrinol (Paris). 2014; 75: 220-226.
14. Barbé, F., Klein, M., Chango, A., Georges, G., Weryha, G., Nicolas, J. P. Homocysteine, folate, vitamin B12, and transcobalamins in patients undergoing successive hypo- and hyperthyroid States. The Journal of Clinical Endocrinology & Metabolism. 2001; 86(4): 1845-1846. https://doi.org/10.1210/jcem.86.4.7436-14
15. Orzechowska-Pawilojc, A., Sworczak, K., Lewczuk, A., Babinska, A. Homocysteine, folate and cobalamin levels in hypothyroid women before and after treatment. Endocr J. 2007; 54(3): 471-476.
16. Gyftaki, H., Kesse-Elias, M., Koutras, D., Pandos, P., Papazoglou, S., Moulopoulos, S. Serum vitamin B12 and folic acid levels in hyperthyroidism. Nuklearmedizin. 1979; 18(6): 278-282.
17. Barchetta, I., Baroni, M. G., Leonetti, F., De Bernardinis, M., Bertoccini, L., Fontana, M. TSH levels are associated with vitamin D status and seasonality in an adult population of euthyroid adults. Clin Exp Med. 2015; 15 (3): 389-396. doi: 10.1007/s10238-014-0290-9
18. Zhang, Y., Wang, Q., Li, Q., Ping Lu, P. Association between hyperhomocysteinemia and thyroid hormones in euthyroid diabetic subjects. Biomed Res Int. 2015; 2015: 196379. doi: 10.1155/2015/196379
19. Kriebitzsch, C., Verlinden, L., Eelen, G. 1,25-Dihydroxyvitamin D3 influences cellular homocysteine levels in murine preosteoblasticMC3T3–E1 cells by direct regulation of cystathionine beta-synthase. J Bone Miner Res. 2011; 26(12): 2991-3000.
20. Muhammad, A., Rehan, Q. Homocysteine in asymptomatic adults. J Clin Endocrinol Metab. 2014; 99: 633-638.
21. Afsaneh, T., Fariba, G., Zatollah, A. The effects of vitamin D supplementation on thyroid function in hypothyroid patients: A randomized, double blind placebo controlled trail. Indian J of Endocrinology & Metabolism. 2018; 22(5): 584-588.
22. Adam, M., Michal, J. K., Waldemar, H., Adam, C., Marta, K., Ryszard, Z., *et al.*, Vitamin D receptor gene polymorphism and autoimmune thyroiditis: Are they associated with disease occurrence and its features? Biomed Research International. 2019; 2019: 1-9 https://doi.org/10.1155/2019/8197580.
23. Tamer, G., Arik, S., Tamer, I., Coksert, D. Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid. 2011; 21(8): 891-896.