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A Case Control Study to Evaluate the Levels of Vitamin D, Calcium, Parathyroid Hormone, Phosphate, and Demographic Characteristics of Patients with Hypothyroidism.

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Abstract

Background

Hypothyroidism is an important thyroid disorder resulted due to an imbalance in thehypothalamus-pituitary-thyroid axis and associated with inadequate secretion of the thyroid stimulating hormones (TSH). Due to increasing prevalence, hypothyroidism has become a public health problem worldwide. A very few studies have evaluated the status of Vitamin D, calcium, phosphate, and parathyroid hormone (PTH) in hypothyroidism.

Objectives

This study was aimed to evaluate the blood levels of Vitamin D, calcium, phosphate, and parathyroid hormone (PTH) in patients with hypothyroidism and compare with healthy controls.**Methods**

Vitamin D, Ca, Ph, and PTH levels were estimated in the serum samples by Electro-chemiluminescence method using Cobas e411 from Roche Company.Patients with hypothyroidism (n=195) referred to the Endocrine and Diabetes Center lab, Endocrine and Diabetes Center in Nassiriyahfor investigations were recruited in the study. Healthy controls (n=205) were those who came for periodic regular follow-up.

Results

There were 24 (12.3%) males and 171 (87.7 %) females among the patients.The mean age of the patients was 39.12±12.26 years. There was no statistically significant difference in the age and BMI between the cases and the controls. The serum Vit D and Ca levels were significantly lower in the patients as compared to the controls ($p<0.001$). However, there was no statistically significant difference in the serum Phosphate levels between the patients and controls ($p=0.32$). The serum PTH levels, however, was significantly higher in the patients as compared to the controls ($p<0.001$).There was no significant difference in the gender distribution between the patients and the controls ($p=0.87$). Occupation, BMI categories ($p=0.04$), Vitamin D ($p<0.001$), Calcium ($p<0.001$), Phosphate ($p=0.004$), and PTH ($p=0.004$) status significantly varied between cases and controls. The previously diagnosed patients with a mean duration of disease, 27.83 (minimum=2, maximum=144)monthshad significantly higher levels of Vitamin D ($p=0.01$), calcium ($p=0.003$), and phosphate levels ($p=0.004$) as compared to the newly diagnosed patients.

Conclusion

Hypothyroidism is associated withhypovitaminosis D, hypocalcemia, and elevated PTH levels.Therefore, patients with hypothyroidism should be regularly evaluated for the levels of Vitamin D, calcium, and PTH for better management of secondary complications in hypothyroidism.

Keywords: Thyroid, hypothyroidism, Vitamin D, Calcium, Phosphate, Parathyroid hormone (PTH)

Introduction

Thyroid diseases contribute a major proportion in the endocrine disorders worldwide.The prevalence of hypothyroidism and subclinical hypothyroidismis 4-5% and 4-15% respectively [1-3]. Hypothyroidism with a subtle onset and non-specific symptoms makes it difficult for early detection in adults.

The common symptoms of hypothyroidism are fatigue, lethargy,weakness, insomnia, and loss of memory. These symptoms do not have a very high diagnostic value as most of the may be age related factors. This has increased the demand of the thyroid function test for the diagnosis of hypothyroidism over the last 20 years [4]. However, there exists a contradiction in the views on the diagnostic accuracy and criteria. Some believe that biochemical tests are the standard to diagnose hypothyroidism and some believe that biochemical tests can be misleading and diagnosis can be made on the basis of clinical symptoms alone [5, 6].

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Hypothyroidism is caused due to a deficiencyin the thyroid hormones. Decreased levels of thyroid hormones result in reduction in metabolic rates [7]. Hypothyroidism is caused by both pathology of the thyroid gland and due to disorders of the pituitary gland or hypothalamus. Over99.5% of cases of thyroid gland failure results from pathology of the thyroid gland and a very less percentage of cases (<0.5%) results are related with pituitary gland or hypothalamus[8, 9].The condition when the serum thyroid stimulating hormone (TSH) levels are above theupper limit of the reference range but the serum free thyroxine(T4) level is well within the reference range is called subclinical hypothyroidism (SCH). SCH is observed in 3% to 8% ofthe general population. Women show high prevalence of SCH as compared to men. The prevalence of SCH increases with age [10].Another condition of hypothyroidism when the serum TSH levels are elevated but the serumfree thyroxin (T4) level is below the reference range is called overt hypothyroidism [11]. The progressionof SCH to overt hypothyroidism is reported to be about 2–5% annually [12].Thyroid hormones affect almost all the systems of the body. As a result deregulation of thyroid hormones result in multi organ dysfunction.

The most common cause of hypothyroidism is iodine deficiency due to poor iodine content in the diet. In countries where the iodine intake is adequate, the most common cause of hypothyroidism is the autoimmunecondition Hashimoto's thyroiditis[13]. Other less frequent causes of hypothyroidism are radioactive iodine treatment,pathology of the hypothalamus or the anterior pituitary gland,dysfunctional thyroid gland due to congenital defects and previous thyroid surgery.

Hypothyroidism is a common problem worldwide. However, it is very common in the UK. Almost 33.33% of world population lives in countries that are iodine-deficient [14] and the major cause of hypothyroidism in these areas is iodine deficiency. The prevalence of hypothyroidism in countries that are iodine-sufficient ranges from 1% to 2%[15, 16]. It is even more prevalent at 7% among individuals in the age group of 85 and 89 years [17]. Hypothyroidism is more prevalent in women as compared to men (about 10 times higher) [15] .The overt hypothyroidism has a prevalence ranging from 0.2% to 5.3% in Europe[18],[19] and from 0.3% to 3.7% in the USA [20].

The overall prevalence of hypothyroidism was found to be 4.6% in the NHANES III study [1]. Data on the incidence of hypothyroidism in Middle Eastern countries are limited. One systematic review [21]evaluated 21 studies that addressed thyroid disease prevalence across ten Middle Eastern countries; however, there was wide heterogeneity in the populations studied, and most of the available studies were convenience samples sourced from cohorts of patients with diabetes mellitus, thyroid cancer or surgical and histopathological series, all of which include patients who are at high risk of thyroid dysfunction. In Tehran, an iodine-sufficient area of Iran, the annual incidence rates of subclinical and overt hypothyroidism were 7.62 and 2.0 per 1,000 persons, respectively [22], and in the same population, thyroid antibodies were detected in 16% of women and 8% of men [23], figures that are comparable to data from European populations [24].

Thyroid hormones are the key players in several physiological mechanisms in the body. Most importantly, the thyroid hormones regulate several physiological activities such as the metabolic the electrolytes and minerals balance in the cells. Therefore, any change in the thyroid hormone levels has serious consequences on the homeostasis of these electrolytes such as Ca, K, and Ph. Studies have reported decreased total calcium and ionised calcium in the patients with hypothyroidism as compared to patients with euthyroid status.

Ca being an important mineral necessary for the teeth and bones formation, muscle contraction, blood coagulation, several enzymatic activities, and normal cardiac functioning, its dysregulation in hypothyroidism should be evaluated in the patients and managed accordingly [25].. Another important element that is dysregulated in hypothyroidism is Vit D. Studies have shown that hypothyroidism patients show hypovitaminosis D and both hypocalcaemia and hypovitaminosis D correlate with the disease severity in the patients [26, 27].

Hypothyroidism has also been reported to be associated with parathyroid hormone insufficiency (Parathormone, PTH) characterized by hypocalcemia and hypovitaminosis D [28].There are no studies in our population evaluating the clinical profile and levels of Calcium, Vitamin D, PTH, and Phosphate. Therefore, this study was planned to evaluate the levels of Calcium, Vitamin D, PTH, and Phosphate in a cohort of patients with hypothyroidism.

Materials and Methods

The case control study was performed in the Endocrine and Diabetes Center in Nassiriyah for a period of 6 months (1st of October 2019 till the end of March 2020).Patients with hypothyroidism referred to the Endocrine and Diabetes Center lab for investigations were recruited in the study. Both the newly diagnosed cases and those who attended the clinic for follow-up were included in the study. While controls were those who attended for periodic

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regular follow-up. The demographic data and the lab results were collected directly from the cases and the controls and entered in a pre-designed proforma.

iochemical tests

Vitamin D, Ca, Ph, and PTH levels were estimated in the serum samples by Electro-chemiluminescence method using Cobas e411 from Roche Company. Manufacturer's protocol was strictly followed for the evaluation of the levels of these analytes. Vitamin D levels were evaluated by measuring total 25-hydroxy vitamin D in the serum and the patients were divided into 3 groups on the basis of vitamin D levels: sufficient (30-64 ng/ml), insufficient (18-29 ng/ml), and deficient (<18 ng/ml). Parathyroid hormone normal level was (15-65 Pg/ml) according to the same protocol, while those for Calcium and Phosphorus were (8.5-10.5 mg/dl) and (2.5-4.5 mg/dl) respectively.

Statistical analysis

Statistical analysis was done by SPSS program (version 21.0, SPSS Inc., Chicago, IL). Normality of the data distribution was checked by Shapiro-Wilk test. The continuous variables were presented as mean±SD and categorical variables were presented as frequency (%). Continuous variables were compared by student's t test and categorical variables were compared by Chi-Square test. Pearson's correlation analysis was performed to evaluate the correlation among different variables. P values less than 0.05 were considered to be statistically significant.

Results

After applying strict inclusion and exclusion criteria and upon receiving signed consent forms, 195 patients with hypothyroidism and 205 healthy controls were included in the study. There were 24 (12.3%) males and 171 (87.7%) females among the patients.The mean age of the patients was 39.12±12.26 years. The mean BMI of the patients was 29.95±3.60 kg/m2. The mean serum Vit D, Ca, Ph, and PTH was 11.95±5.73, 8.88±0.46, 3.44±0.55, and 48.81±16.09 respectively. There was no statistically significant difference in the age and BMI between the cases and the controls. The serum Vit D and Ca levels were significantly lower in the patients as compared to the controls ($p<0.001$). However, there was no statistically significant difference in the serum Ph levels between the patients and controls ($p=0.32$). The serum PTH levels, however, was significantly higher in the patients as compared to the controls ($p<0.001$) (Table 1).

Table 1: Comparison of continuous variables between cases and controls. Data represented as Mean±SD. Difference of mean is considered to be statistically significant at p values<0.05.

	Case (n=195)	Control (n=205)	p value
Age (Mean±SD)	39.12±12.26	38.13±10.77	0.31
BMI (Mean±SD)	29.95±3.60	29.41±4.12	0.18
VitD (Mean±SD)	11.95±5.73	25.48±8.25	<0.001
Ca (mg/dl) (Mean±SD)	8.88±0.46	9.11±0.41	<0.001
Ph (mg/dl) (Mean±SD)	3.44±0.55	3.39±0.41	0.32
PTH (Mean±SD)	48.81±16.09	43.69±5.58	<0.001

There was no significant difference in the gender distribution between the patients and the controls ($p=0.87$). There was a statically significant difference in the occupation between the patients and the controls ($p=0.01$).There was a significant difference in the BMI categories ($p=0.04$). There were significantly higher number of grade I and grade II obese individuals among the patients as compared to the controls. However, the number of overweight individuals was significantly higher in the controls as compared to the patients. There was significantly higher number of individuals with Vit. D deficiency among the patients as compared to the controls ($p<0.001$). There was significantly higher number of individuals with low Ca levels among the patients as compared to the controls

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(p<0.001). There was significantly higher number of individuals with low Ph levels among the patients as compared to the controls (p=0.004). There were 13 (7.2%) patients with high PTH levels whereas there were none in the controls (Table 2).

Table 2: Comparison of the categorical variables between cases and controls. Data is represented as frequency (%). Chi-square test was performed to compare the categorical variables between cases and controls. Difference is considered to be statistically significant at p values<0.05.

2.1. Gender					
			no		**p value**
			Case (n=195)	Control (n=205)	
	Gender	Males	24 (11.1%)	45 (10.3%)	**0.87**
		Females	171 (88.9%)	160 (89.7%)	

2.2. Occupation				
		no		p value
		Case (n=195)	Control (n=205)	
Occupation	housewife	132 (67.7%)	102 (49.7%)	0.01
	employer	18 (9.2%)	46 (22.4%)	
	engineer	2 (1%)	4 (2%)	
	teacher	8 (4%)	13 (6.3%)	
	medical staff	3 (1.5%)	2 (1.0%)	
	policeman	0 (0.0%)	2 (1.0%)	
	student	14 (7.2%)	22 (10.7%)	
	self employed	14 (7.2%)	14 (6.8%)	
	retired	3 (1.5%)	0 (0.0%)	
	Biologist	1 (0.5%)	0 (0.0%)	

2.3. BMI Category				
		no		p value
		Case (n=195)	Control (n=205)	
BMICat	Normal	11 (5.6%)	12 (5.9%)	0.04
	Overweight	92 (47.1%)	126 (61.4%)	
	Grade I obesity	72 (37 %)	51 (24.9%)	
	Grade II obesity	17 (8.7%)	12 (5.9%)	
	Grade III morbid obesity	3 (1.5%)	4 (2.0%)	

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2.4. Vit D Category				
		no		p value
		Case (n=195)	Control (n=205)	
VitDCat	Deficient	167 (85.6%)	42 (20.5%)	<0.001
	Insufficient	26 (13.3%)	102 (49.7%)	
	Sufficient	2 (1.1%)	61 (29.7%)	

2.5. Calcium Category				
		no		Total
		Case (n=195)	Control (n=205)	
CaCat	Normal	165 (84.6%)	202 (98.5%)	<0.001
	High	1 (0.5%)	0 (0%)	
	Low	29 (14.9%)	3 (1.5%)	

2.6. Phosphate Category				
		no		p value
		Case (n=195)	Control (n=205)	
PhCat	Normal	182 (93.3%)	203 (99.0%)	0.004
	Low	13 (6.7%)	2 (1.0%)	

2.7. PTH Category				
		no		p value
		Case (n=195)	Control (n=205)	
PTHCat	Normal	181 (92.8%)	205 (100.0%)	<0.001
	High	14 (7.2%)	0 (0.0%)	

Vitamin D levels were negatively correlated with BMI (r=-0.248, p=0.001) and PTH (r=-0.326, p<0.001), positively correlated with Calcium levels (r=0.431, p=0.001). Calcium levels were positively correlated with age (r=0.237, p=0.001), vitamin D (r=0.431, p=0.001), and Phosphate (r=0.148, p=0.047) while negatively correlated with PTH levels (r=-0.350, p<0.001) in the patients. Only the phosphate levels were positively correlated with the duration of disease (r=0.275, p<0.001) (Table 3).

Table 3: Pearson's correlation analysis in the cases. P vale<0.05 is considered to be significant.

Pearson's Correlations (Cases)							
	Age	BMI	Vitamin D	Calcium	Phosphate	PTH	Durationofhypothyroidism

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age	Pearson Correlation	1	**.377****	.101	**.237****	.126	.064	.132
	Sig. (2-tailed)		**.000**	.175	**.001**	.093	.394	.078
BMI	Pearson Correlation	**.377****	1	**-.248****	-.080	.039	**.283****	-.013
	Sig. (2-tailed)	**.000**		**.001**	.286	.605	**.000**	.867
Vitamin D	Pearson Correlation	.101	**-.248****	1	**.431****	.130	**-.326****	.035
	Sig. (2-tailed)	.175	**.001**		**.000**	.082	**.000**	.643
Calcium	Pearson Correlation	**.237****	-.080	**.431****	1	**.148***	**-.350****	.051
	Sig. (2-tailed)	**.001**	.286	**.000**		**.047**	**.000**	.499
Phosphate	Pearson Correlation	.126	.039	.130	**.148***	1	.102	**.275****
	Sig. (2-tailed)	.093	.605	.082	**.047**		.174	**.000**
PTH	Pearson Correlation	.064	**.283****	**-.326****	**-.350****	.102	1	-.067
	Sig. (2-tailed)	.394	**.000**	**.000**	**.000**	.174		.370
Durationofhypothyroidism	Pearson Correlation	.132	-.013	.035	.051	**.275****	-.067	1
	Sig. (2-tailed)	.078	.867	.643	.499	**.000**	.370	
**. Correlation is significant at the 0.01 level (2-tailed).								
*. Correlation is significant at the 0.05 level (2-tailed).								

There were 53 newly diagnosed cases and 142 previously diagnosed cases of hypothyroidism. The mean duration of hypothyroidism at the time of recruitment was 27.83 months (minimum=2, maximum=144 months). The previously diagnosed patients had significantly higher levels of Vitamin D (p=0.01), calcium (p=0.003), and phosphate levels (p=0.004) as compared to the newly diagnosed patients (Table 4).

Table 4:Comparison of different biochemical parameters between newly diagnosed and previously diagnosed cases. Data is represented as mean±SD. Student's t test was performed to compare the variables between groups. Difference is considered to be statistically significant at p values<0.05.

	Newly diagnosed cases (n=53)	Old cases (n=142)	p value
VitD (Mean±SD)	**10.21±4.31**	**12.68±6.09**	**0.01**
Ca (mg/dl) (Mean±SD)	**8.72±0.46**	**8.95±0.45**	**0.003**

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Phosphate (mg/dl) (Mean±SD)	**3.25±0.58**	**3.51±0.51**	**0.004**
PTH (Mean±SD)	50.05±14.95	48.28±16.57	0.50

Discussion

The present study included 195 patients with hypothyroidismconducted at Endocrine and Diabetes Center in Nassiriyah for a period of 6 months (1st of October 2019 till the end of March 2020). The proportion of females was higher as compared to males among the hypothyroidism patients. There were 171 (87.7%) females and 24 (12.3%) resulting in a female to male ratio of 7:1. Similar to the findings of the present study higher prevalence of hypothyroidism in females has been reported in earlier studies[29-32].

Hypothyroidism occurs as a result of an imbalance in the hypothalamus-pituitary-thyroid axis and inadequate secretion of the thyroid stimulating hormones (TSH) [33]. Hypothyroidismhas several etiologies; however, the most common etiologies are Hashimoto or autoimmune thyroid disorders and iodine deficiency[34]. Several studies have shown the role of Vitamin D in the prevention of autoimmune thyroid disease. It has been reported that Vitamin D ameliorates abnormal immune responses by acting on the immune cells [35]. However, Vitamin D has also been seen to affect the thyroid function other than modulating the immunecells in autoimmune diseases. A very few studies have evaluated the role of Vitamin D in non-immune hypothyroidism disease.A study has shown that vitamin D deficiency (< 20 ng/mL) was more prevalent in patients with hypothyroidism as compared to healthy controls[5]. Inanother study it was shown that vitamin D deficiency was more common in patientswith Hashimoto than healthy control [36]. Several similar studies support this association of Vitamin D deficiency with hypothyroidism [37-39]. In agreement to these studies we also observed that the patients with hypothyroidism had lower Vitamin D levels as compared to healthy controls and the prevalence of Vitamin D deficiency was higher in the patients with hypothyroidism than the healthy controls.

It has been shown that Vitamin D supplementation can positively affect the prognosis of hypothyroidism[40].In a study it has been shown that TSH levels are negatively correlated with Vitamin D levels[41, 42]. In the present study we did not evaluate the relationship of TSH levels with Vitamin D levels as all the patients were on varying doses of Levothyroxine which would affect the levels of TSH. This would affect the interpretation of TSH and Vitamin D levels.In a study it has been reported that Levothyroxine raised the Vitamin D levels in patients with hypothyroidism [43]. In the present study it was observed that the newly diagnosed patients had lower Vitamin D levels as compared to previously diagnosed patients. This might be explained in terms of the Vitamin D rising effects of Levothyroxine in the previously diagnosed patients. The findings of the present study warrant for the regular evaluation of Vitamin D levels in patients with hypothyroidism.

FT3 and FT4 stimulate osteoblastic differentiation in the bones and subsequently promotebone resorption and hence result in an increase in the blood calcium levels. Reduced levels of FT4 and FT3 levels in patients with hypothyroidism result in impairment of calcium mobilization and hence lea a decrease in blood calcium levels [44]. A study conducted on patients with hypothyroidism reported decreased blood calcium levels (total calcium and ionic calcium) in the patients as compared to the healthy controls [45]. Other studies also supported this observation and had reported decreased blood calcium levels in patients with hypothyroidism as compared to controls[46-48]. In accordance to these studies we also observed lower blood calcium levels in the patient cohort as compared to the healthy controls.There was significantly higher number of individuals with low Ca levels among the patients as compared to the controls.Also, in the present study it was observed that the newly diagnosed patients had lower calcium levels as compared to previously diagnosed patients. Some studies have highlighted contradictory findings where blood calcium levels were reported to be normal in the patients with hypothyroidism [49, 50]. Another study reported no correlation of calcium levels with T3, T4, and TSH in patients with hypothyroidism [51]. However, in the present study it was observed that PTH levels were significantly higher in the patients as compared to healthy controls. Thirteen (7.2%) patients had high PTH levels whereas none of the controls had high PTH levels. No significant difference was observed in the levels of PTH between the newly diagnosed cases and the previously diagnosed hypothyroidism cases. This might be due to an increased release of PTH hormone due to sustained hypocalcemia. Another reason of increased PTH levels in the patients with hypothyroidism is the increase in the biological half life of PTH in hypothyroidism [52]. Thyroid hormones play an important role in homeostasis of Calcium and Phosphorous levels by their direct action on bone turnover. Higher serum Phosphorous levels have been reported in the patients with hypothyroidism as compared to healthy controls [53]. Although in the present

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study serum phosphorous levels were significantly higher in the newly diagnosed cases as compared with the previously diagnosed cases of hypothyroidism, no significant difference in the phosphorous levels were seen between the cases and controls. Significantly higher number of patients had high phosphorous levels as compared to healthy controls.The major limitations of the present study were that we did not evaluate the association of elemental iron and iodine levels among the cases. These elements should be evaluated further and correlated with the parameters studied in the present study. However, the present study provides a comprehensive evaluation of vitamin D, calcium, phosphate, and PTH in hypothyroidism patients that will be useful for the clinicians in the management of the hypothyroidism.

Conclusion

Hypovitaminosis D, hypocalcemia, and elevated PTH levels are significant features of hypothyroidism. Hence, the patients with hypothyroidism should be regularly evaluated for the levels of Vitamin D, calcium, and PTH for better management of secondary complications in hypothyroidism.

References

1. Hollowell JG, Staehling NW, Flanders WD, Hannon WH, Gunter EW, Spencer CA, et al. Serum TSH, T4, and thyroid antibodies in the United States population (1988 to 1994): National Health and Nutrition Examination Survey (NHANES III). J ClinEndocrinolMetab. 2002; 87(2):489-99.
2. Hoogendoorn EH, Hermus AR, de Vegt F, Ross HA, Verbeek AL, Kiemeney LA, et al. Thyroid function and prevalence of antithyroperoxidase antibodies in a population with borderline sufficient iodine intake: Influences of age and sex. Clin Chem. 2006; 52(1):104-11.
3. BEDJARGI, PRUTHVI RAJ C., and RS KULKARNI. "SDS-PAGE ANALYSIS AND ELECTRON MICROSCOPY OF CORPUSCLES OF STANNIUS SECRETION IN THE FRESHWATER FISH, NOTOPTERUS NOTOPTERUS."International Journal of General Medicine and Pharmacy (IJGMP) 3.3, May 2014, 1-6
4. Bemben DA, Hamm RM, Morgan L, Winn P, Davis A, Barton E. Thyroid disease in the elderly. Part 2. Predictability of subclinical hypothyroidism. J Fam Pract. 1994; 38(6):583-588
5. Hadlow, N. C. et al. The relationship between TSH and free T4 in a large population is complex and nonlinear and differs by age and sex. J. Clin. Endocrinol. Metab. 98, 2936–2943 (2013).
6. Salman, Mazin S., and Zaineb M. Jumah. "VITAMIN D LEVEL AMONG SAMPLE OF BASRA PEOPLE."International Journal of General Medicine and Pharmacy (IJGMP) 9.6, Oct–Nov 2020; 1-4
7. Lindsay RS, Toft AD. Hypothyroidism. Lancet. 1997; 349(9049):413-417.
8. Weetman AP. Hypothyroidism: screening and subclinical disease. BMJ. 1997; 314(7088):1175-1178.
9. Gull, W. W. A Collection of the Published Writings of William Wilhey Gull. London, New Sydenham Society, 1894, vol. 1, pp. 315-321.
10. N. Kochupillai. Clinical endocrinology in India. Current science, vol. 79(8) october 2000.
11. Kenneth A.Woeber, MD, FRCPE. Update on the Management of Hyperthyroidism and Hypothyroidism.
12. VahabFatourechi, MD: Subclinical Hypothyroidism: An Update for Primary Care Physicians, mayo Clin Proc. Jan 2009; 84(1): 65–71
13. Pearce, S. H. et al. 2013 ETA guideline: management of subclinical hypothyroidism. *Eur.* Thyroid J. 2, 215–228 (2013).
14. Melissa Gillett, Subclinical Hypothyroidism: Subclinical Thyroid Disease: Scientific Review and Guidelines for Diagnosis and Management JAMA 2004;291: 228–38
15. Chaker, L., Bianco, A. C., Jonklaas, J. &Peeters, R. P. Hypothyroidism. *Lancet* **390**, 1550–1562 (2017).
16. Chakrabarti, A. S. I. T., et al. "Effect of sweet potato based ration on physiological and blood profile of broiler rabbit." *International J Botany Res. 7 (1): 1* 18 (2017).
17. Zimmermann, M. B. Iodine deficiency. *Endocr. Rev.* **30**, 376–408 (2009).
18. Vanderpump, M. P. The epidemiology of thyroid disease. *Br. Med. Bull.* **99**, 39–51 (2011).
19. Parle, J. V., Franklyn, J. A., Cross, K. W., Jones, S. C. & Sheppard, M. C. Prevalence and follow-up of abnormal thyrotrophin (TSH) concentrations in the elderly in the United Kingdom. *Clin. Endocrinol.* **34**, 77–83 (1991).
20. Gussekloo, J. et al. Thyroid status, disability and cognitive function, and survival in old age. JAMA 292, 2591–2599 (2004).
21. Asvold, B. O., Vatten, L. J. &Bjoro, T. Changes in the prevalence of hypothyroidism: the HUNT Study in Norway. Eur. J. Endocrinol. 169, 613–620 (2013).

22. AL-DHAFEERI, NAWAF M. "LEARNING DISABILITIES RELATIONSHIP WITH SOME BIOLOGICAL FACTORS-COMPARATIVE STUDY–."International Journal of Educational Science and Research (IJESR) 3.5, Dec 2013, 43-52
23. McGrogan, A., Seaman, H. E., Wright, J. W. & de Vries, C. S. The incidence of autoimmune thyroid disease: a systematic review of the literature. Clin. Endocrinol. 69, 687–696 (2008).
24. Canaris, G. J., Manowitz, N. R., Mayor, G. & Ridgway, E. C. The Colorado thyroid disease prevalence study. Arch. Intern. Med. 160, 526–534 (2000).
25. Al Shahrani, A. S. et al. The epidemiology of thyroid diseases in the Arab world: a systematic review. *J. Public Health Epidemiol.* **8**, 17–26 (2016).
26. Amouzegar, A. et al. Natural course of euthyroidism and clues for early diagnosis of thyroid dysfunction: Tehran Thyroid Study. Thyroid 27, 616–625 (2017).
27. Amouzegar, A. et al. The prevalence, incidence and natural course of positive antithyroperoxidase antibodies in a population-based study: Tehran Thyroid Study. PLOS ONE 12, e0169283 (2017).
28. Knudsen, N., Jorgensen, T., Rasmussen, S., Christiansen, E. &Perrild, H. The prevalence of thyroid dysfunction in a population with borderline iodine deficiency. Clin. Endocrinol. 51, 361–367 (1999).
29. Goltzman D. Clinical manifestations of hypocalcemia. Available at: https://somepomed. org/articulos/contents/mobipreview.htm?39/49/40734?source=see_link
30. Theodore C. Friedman. Vitamin D Deficiency and Thyroid Disease. www.goodhormonehealth.com/VitaminD.
31. Ahi S, Dehdar MR, Hatami N. Vitamin D deficiency in non-autoimmune hypothyroidism: a case-control study. BMC EndocrDisord. 2020 Mar 20;20(1):41. doi: 10.1186/s12902-020-0522-9. PMID: 32192469; PMCID: PMC7082994.
32. KUMAR, NIRAJ, et al. "PSEUDO HYPOPARATHYROIDISM PRESENTING WITH REFRACTORY SEIZURES: AN INTERESTING REPORT OF 3 CASES."International Journal of Medicine and Pharmaceutical Sciences (IJMPS)5.3, Jun 2015, 59-64
33. Mantovani G, Elli FM, Corbetta S. Hypothyroidism associated with parathyroid disorders. Best Pract Res ClinEndocrinolMetab. 2017 Mar;31(2):161-173. doi: 10.1016/j.beem.2017.04.004. Epub 2017 Apr 15. PMID: 28648505.
34. Crowley WF, Ridgway C, Bough EW, Francis GS, Daniels GH, Kourides IA, et al. Noninvasive evaluation of cardiac function in hypothyroidism. N Engl J Med. 1977; 296(1):1-6.
35. Jagdish HS, Batra A, Siwach SB, Kumar R, Gupta R. An Echocardiographic Study on the Effect of Levothyroxine Therapy of Cardiac Function and Structure in Hypothyroidism. JIACM. 2009;10(1&2):27-31.
36. Shah SKD, Kilari M, Shah NKS. Cross sectional study of cardiovascular manifestations of hypothyroidism. J Evolution Med Dental Sci 2013;2(27):5021-5029.
37. Dixit R, Kumar A, Tripathi DK, Dwivedi KK. Study the Clinical Profile of Hypothyroidism in a Tertiary Care Hospital. Int J Sci Res. 2015;4(2):273-276.
38. Gaitonde DY, Rowley KD, Sweeney LB. Hypothyroidism: an update. S Afr Fam Pract. 2012;54(5):384–90.
39. Garber JR. Woeber for the American Association of Clinical Endocrinologists and American Thyroid Association Taskforce on hypothyroidism in adults KA. Clinical practice guidelines for hypothyroidism in adults: cosponsored by the American Association of Clinical Endocrinologists and the American Thyroid Association. Thyroid. 2012;22:1200–35.
40. Chambers ES, Hawrylowicz CM. The impact of vitamin D on regulatory T cells. Curr Allergy Asthma Rep. 2011;11(1):29–36.
41. Evliyaoğlu O, Acar M, Özcabı B, Erginöz E, Bucak F, Ercan O, Kucur M. Vitamin D deficiency and Hashimoto's thyroiditis in children and adolescents: A critical vitamin D level for this association? J Clin Res PediatrEndocrinol. 2015;7(2):128.
42. Tamer G, Arik S, Tamer I, Coksert D. Relative vitamin D insufficiency in Hashimoto's thyroiditis. Thyroid. 2011;21(8):891–6.
43. Bozkurt N, Karbek B, Ucan B, Sahin M, Cakal E, Ozbek M, Delibasi T. The association between severity of vitamin D deficiency and Hashimoto's thyroiditis. EndocrPract. 2013;19(3):479–84.
44. Ucan B, Sahin M, SaykiArslan M, Colak Bozkurt N, Kizilgul M, Güngünes A, Cakal E, Ozbek M. Vitamin D treatment in patients with Hashimoto's thyroiditis may decrease the development of hypothyroidism. Int J VitamNutr Res. 2017;1:9.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 3143 - 3152
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45. Krysiak R, Szkróbka W, Okopień B. The effect of vitamin D on thyroid autoimmunity in levothyroxine-treated women with Hashimoto's thyroiditis and normal vitamin D status. ExpClinEndocrinol Diabetes. 2017;125(04):229–33.
46. Colbay M, Altay M, Akturk M, Cakir N, Yetkin I, Arslan M. Vitamin D levels are associated with serum TSH levels but not with thyroid autoantibodies. In: 13th European Congress of Endocrinology, BioScientifica. 2011;26.
47. Zhang Q, Wang Z, Sun M, Cao M, Zhu Z, Fu Q, Gao Y, Mao J, Li Y, Shi Y, Yang F. Association of high vitamin d status with low circulating thyroidstimulating hormone independent of thyroid hormone levels in middleaged and elderly males. Int J Endocrinol. 2014;2014:631819.
48. Bársony J, Lakatos P, Földes J, Fehér T. Effect of vitamin D3 loading and thyroid hormone replacement therapy on the decreased serum 25-hydroxyvitamin D level in patients with hypothyroidism. ActaEndocrinol. 1986;113(3):329–34.
49. Tuchendler D, Bolanowski M. The influence of thyroid dysfunction on bone metabolism. Thyroid Res. 2014;7:12.
50. Mani V, Natarajan MSS, Mohankrishnan VV. Comparison of Total and Ionic Calcium in Hypothyroidism. Journal of Clinical and Diagnostic Research. 2019 May, Vol-13(5): BC10-BC12DOI: 10.7860/JCDR/2019/39521.12842
51. Shivaleela MB, Poornima RT, Jayaprakash Murthy DS. Serum calcium and phosphorous levels in thyroid dysfunction. Indian Journal of Fundamental and Applied Life Science 2012; 2(2):179-83.
52. Gohel MG, Shah AM, Shah AM, Makadia JS. A study of serum calcium, [15] magnesium and phosphorus level in hypothyroidism patients. Int J Med Health Sci. 2014;3(4):308-12.
53. Abdel-Gayoum AA. Dyslipidemia and serum mineral profiles in patients with [16] thyroid disorders. Saudi Med J. 2014;35(12):1469-76.
54. Begic-Karup S, Wagner B, Raber W, Schneider B, Hamwi A, Waldhäusl W. Serum calcium in thyroid disease. Wein KlinWochenschr. 2001;113(1-2):65-8.
55. Sabuncu T, Aksoy N, Arikan E, Ugur B, Tasan E, Hatemi H. Early changes in the parameters of bone and mineral metabolism during therapy for hyperthyroidism and hypothyroidism. Endocrine Research. 2001; 27(1- 2):201-13.
56. Susanna TY, Sagayaraj A, Shashidhar KN, Gomathi M, Mahesh V. A correlative study of thyroid profile and mineral status in patients with hypothyroidism-A hospital-based case control study. Asian J Pharm Clin Res. 2016;9(3):292-4.
57. Emer O, KaracaliogluAO, Ince S, Alagöz E, Gunalp B and Arslan N. Does increase in TSH levels affect PTH levels? Journal of Nuclear Medicine May 2014, 55 (supplement 1) 1922.
58. AlaEldin S. Ashmaik, Haala M. Gabra, Amna O M. Elzein, NassrEldin M A.Shrif, Elhashimi E. Hassan. Assessment of Serum Levels of Calcium and Phosphorous in Sudanese Patients with Hypothyroidism. Asian Journal of Biomedical and Pharmaceutical Sciences; 03 (25); 2013; 21-26.