Article

Comparison of Vitamin D Levels, Bone Metabolic Marker Levels, and Bone Mineral Density among Patients with Thyroid Disease: A Cross-Sectional Study

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Abstract: Thyroid hormones have a catabolic effect on bone homeostasis. Hence, this study aimed to evaluate serum vitamin D, calcium, and phosphate and bone marker levels and bone mineral density (BMD) among patients with different thyroid diseases. This cross-sectional study included patients with underlying thyroid diseases (n=64, hyperthyroid; n=53 euthyroid; and n=18, hypothyroid) and healthy controls (n=64). BMD was assessed using z-score and left hip and lumbar bone density (g/cm²). Results showed that the mean serum vitamin D Levels of all groups was low (<50 nmol/L). Thyroid patients had higher serum vitamin D levels than healthy controls. All groups had normal serum calcium and phosphate levels. The bone marker levels were significantly high in the hyperthyroid group and low in the hypothyroid group. The z-score for hip and spine did not significantly differ between thyroid patients and control groups. The hip bone density was remarkably low in the hyperthyroid group. In all groups, the serum vitamin D levels were below the cutoff value. However, thyroid patients had a higher serum vitamin D level than healthy controls. The carboxy terminal collagen crosslink and procollagen type I N-terminal propeptide levels were high in hyperthyroid patients and low in hypothyroid patients. Further, hyperthyroid patients had a low hip BMD.

Keywords: Hyperthyroid; Hypothyroid; Euthyroid; Vitamin D; Bone Turn Over Markers; Bone Mineral Density

1. Introduction

Based on the incidence of thyroid disorders and thyroid autoantibodies, the prevalence rates of overt and subclinical hypothyroidism were 2.5 and 14.7 per 1000 population, respectively, in individuals living in the coastal communities of Malaysia. Meanwhile, those of overt and subclinical hyperthyroidism were 4.9 and 29.4 per 1000 population, respectively. [1] Thyroid hormones have a direct catabolic effect on bone mineral homeostasis, leading to increased bone mineral resorption and calcium loss via the kidneys. [2] Hormones play an essential role in maintaining homeostasis and regulating bone density. Hyperthyroidism and hypothyroidism are correlated with low BMD and higher risk of fracture. [3] In the study of Maccagnano G et al. (2016), the incidence rates of fragility fractures were 4.5% and 3.7% in individuals with hyperthyroidism and hypothyroidism, respectively. [4]

The Malaysia's clinical practice guideline on the management of osteoporosis, which was published in 2010, had recommended the use of hyperthyroidism as an indicator of low BMD. In India, hyperthyroid patients with concomitant vitamin D deficiency had a lower BMD than those with adequate vitamin D levels. [5] Low calcium and vitamin D levels in this group of patients may increase the risk of osteoporosis. No standard consensus had shown that calcium or vitamin D supplementation is effective for the treatment of thyroid disorders. Calcium and vitamin D deficiencies are a global health problem. Recent studies have shown that vitamin D deficiency is common even in tropical countries, such as Vietnam, Malaysia, and Indonesia. [6-8] In Malaysia, studies have only assessed the vitamin D status of school children, [9] Malay employees of an academic institution, [8] women of child-bearing age, [6] and postmenopausal women. [10] Therefore, this study aimed to evaluate and compare serum vitamin D, calcium, and phosphate and bone turnover marker levels and bone mineral density among patients with different thyroid disorders.

2. Materials and Methods

This cross-sectional study was conducted at the Endocrine Clinic, Hospital Universiti Sains Malaysia (HUSM) and Hospital Raja Perempuan Zainab (2), Kota Bharu, Kelantan, Malaysia. In total, 199 participants (n=135, those with thyroid diseases and n=64, healthy controls) aged between 20 and 40 years were enrolled in this research from June 2017 to July 2018. A written informed consent was obtained from all patients. The study was conducted based on the guidelines for good clinical practice, and the Human Research Ethics Committee of USM (USM/JEPeM/17010025) and Malaysia Registry Ethical Committee (NMRR-16-2477-33191 [IIR]) approved this study.

The inclusion criteria were as follows: patients with hyperthyroidism, biochemically hyperthyroid state for at least 6 months with or without medications; those with hypothyroidism, biochemically hypothyroid state for at least 6 months with or without medications; and those with euthyroid, biochemically euthyroid state for at least 6 months with or without medications. Participants with subclinical diseases were also included in this study. Patients in the control group did not present with any thyroid diseases. The exclusion criteria included individuals with chronic diseases (liver and renal impairment and diabetes); post-menopausal, lactating, and pregnant women; those on medication (OCP, steroids, calcium and vitamin D supplements, and antiepileptic drugs) affecting bone metabolism; and those with chronic gastrointestinal disease, thyroid malignancy, and history of fracture within a year from the study period.

The sample size for each objective was calculated using two population mean formulae[11]. We calculated the sample size based on the method used in previous studies. The calculation for serum 25-hydroxyvitamin D (25(OH)D) levels had the largest sample size.

The records of all patients with thyroid diseases had been assessed a day before follow-up. The most recent thyroid function test result (within 1 month before follow-up) was reviewed. The participants who fulfilled the criteria were contacted, and objectives well as the rationale of the study

was provided. The participants were instructed to fast overnight for 10–12 h prior to blood sampling to minimize variations in bone marker levels. On the appointment day, detailed information regarding the study was provided again to the participants, and blood samples were collected from patients who provided consent. The samples for the assessment of 25(OH)D, calcium, phosphate, procollagen type I N-terminal propeptide (P1NP), and carboxy terminal collagen crosslink (CTX) levels were sent immediately to the laboratory. After centrifugation, the serum was stored at −70°C before analysis.

Serum calcium and phosphorus levels were assessed via a spectrophotometric analysis using the ARCHITECT C800 analyzer (Abbott Diagnostics). The corrected calcium level was calculated using the following formula: corrected calcium (mmol/L)=measured total calcium level (mmol/L)+0.02 (40–serum albumin level [g/L]). The serum 25(OH)D level was evaluated using the paramagnetic particle, chemiluminescent immunoassay competitive principle with the Access 2 Immunoassay System analyzer (Beckman Coulter). This method is traceable to a Joint Committee for Traceability in Laboratory Medicine using isotope dilution mass spectrometry reference method procedure (RMP) developed at Ghent University [12,13]. This RMP is further traceable to the NIST SRM 2972. According to the World Health Organization and the Institute of Medicine, the optimum vitamin D level threshold was 50 nmol/L [14,15]. The participants were classified based on the serum 25(OH)D levels, which were as follows: vitamin D sufficiency (≥50 nmol/L), insufficiency (<50 nmol/L), and deficiency (<30 nmol/L).

The total P1NP's CVs were 1.2%–3.0% and 1.7%–4.1% for repeatability and intermediate precision, respectively. Meanwhile, the CTX's CVs were 2.1%–3.5% and 2.8%–8.4% for repeatability and intermediate precision, respectively. The total P1NP, CTX, TSH, and FT4 levels were assessed using the automated Cobas e601 immunoassay analyzer. A sandwich assay was used to measure the total P1NP and CTX levels. The TSH and FT4 values were evaluated with the electrochemiluminescent assay based on the sandwich and competitive principle.

The left hip and lumbar spine (L1–L4 vertebrae) BMD was assessed using dual-energy X-ray absorptiometry (DEXA) with the Bone Densitometer Hologic Discovery with a fan beam technology (Waltham, the USA). Absolute BMD was the measured parameter using the bone mineral content in grams over a two-dimensional projected area of the bone in cm^2. Thus, the BMD unit was g/cm^2 [14].

The total z-scores of the left hip and lumbar spine were considered as the representative values. The z-score was used to represent a bone density in average individuals of the same age and sex (using Bone Densitometer Hologic Discovery QDR). According to the International Society for Clinical Densitometry 2015, a z-score of ≤−2.0 was below the expected range according to age, and a z-score of >−2.0 was within the expected range based on age.

Data were subjected to statistical analysis using the Statistical Package for the Social Sciences software version 24. For numerical variables, the results were presented as mean and standard deviation (SD) for data with normal distribution or as median and interquartile range (IQR) for skewed data. For categorical variables, the results were presented as frequency (n) and percentage (%). According to the different groups, the baseline characteristics of the participants were compared using one-way analysis of variance (ANOVA) for numerical variables and chi-square test or Fisher's exact test for categorical variables. The difference in each variable between groups was compared using one-way ANOVA, followed by post hoc multiple pairwise comparisons using the Bonferroni procedure if the assumption of homogeneity for variance was fulfilled or the Games-Howell procedure if the assumption of homogeneity for variance was violated. Age and sex were adjusted in the analysis using two-way analysis of covariance (ANCOVA) or factorial ANCOVA, followed by multiple pairwise comparisons using the Bonferroni procedure. A p value <0.05 was considered statistically significant.

3. Results

3. 1 Baseline Characteristics of the Participants According to Group

Patients with thyroid diseases and healthy controls (n=199) participated in this study. This research included euthyroid, hyperthyroid, and hypothyroid patients and healthy controls with a mean age (SD) of 31.77 (5.27), 29.69 (5.95), 28.89 (5.9), and 28.55 (6.17), respectively. The result was statistically significant. Most participants were Malay (n=61 [93.5%], hyperthyroid; n=17 [94.4%], hypothyroid; n=49 [92.5%], euthyroid; and n=60 [93.7%], healthy controls), and only few were Chinese (n=3 [4.7%], hyperthyroid; n=1 [5.6%], hypothyroid; n=4 [7.5%], euthyroid; and n=4 [6.3%], healthy controls). The euthyroid group had a higher body mass index (BMI) (mean [SD]: 25.79 [5.61] kg/m^2) than the hypothyroid (24.43 [6.08] kg/m^2) and hyperthyroid (23.69 [3.92] kg/m^2) groups. The BMI of the control group was 24.44±4.6 kg/m^2.

The duration of illness was longer in the hypothyroid group (mean [SD]: 75.33 [84.31] months) than in the euthyroid (66.26 [63.26] months) and hyperthyroid (42.48 [39.43] months) groups. The mean TSH levels (SD) of the hyperthyroid, hypothyroid, euthyroid, and control groups were 0.16 (0.63), 23.10 (13.89), 2.52 (3.07), and 1.8 (0.84) mIU/L, respectively. Meanwhile, the mean FT4 levels (SD) were 32.87 (17.13), 13.31 (12.3), 17.06 (3.55), and 16.12 (2.18) pmol/L in the hyperthyroid, hypothyroid, euthyroid, and control groups, respectively (Table 1).

Table 1 : Baseline characteristic of patients with thyroid disease and control groups.

Variables	Hyperthyroid	Hypothyroid	Euthyroid	Control	test-stat (df)	P-value
Frequency, n(%)	64 (32)	18 (9)	53 (27)	64 (32)		
Mean Age, years (SD	29.69 (5.95)	28.89 (5.9)	31.77 (5.27)	28.55 (6.17)	3.14 (3, 195)	0.026[a]
Gender						
Male	16 (25)	5 (27.8)	11 (20.8)	27 (42.2)	7.53 (3)	0.057[b]
Female	48 (75)	13 (72.2)	42 (79.2)	37 (57.8)		
Race						
Malay	61 (95.3)	17 (94.4)	49 (92.5)	60 (93.7)	-	0.124[c]
Non Malay	3 (4.7)	1 (5.6)	4 (7.5)	4 (6.3)		
Mean BMI (kg/m^2)	23.69 (3.92)	24.43 (6.08)	25.79 (5.61)	24.44 (4.6)	1.84 (3, 195)	0.141[a]
Mean Duration of Thyroid Disease, Months (SD)	42.58 (39.43)	75.33 (84.31)	66.26 (63.26)	-	3.68 (2, 132)	<0.001[a]
Mean Duration Current State of Thyroid Function, Months (SD)	42.20 (39.02)	38.06 (86.82)	38.25 (43.49)		38.25 (43.49)	0.896[a]
Mean TSH mIU/L (SD)	0.16 (0.63)	23.10 (13.89)	2.52 (3.07)	1.8 (0.84)	133.25 (3, 195)	0.001[a]
MeanFT4 pmol/L (SD)	32.87 (17.13)	13.31 (12.3)	17.06 (3.55)	16.12 (2.18)	36.53 (3, 195)	0.001[a]

Data described as mean (SD) for numerical variables and n (%) for categorical variables.

[a]One way ANOVA p-value

[b]Chi2 test p-value

cFisher exact test p-value

Graves' disease was the most common diagnosis (n=51 [80%], hyperthyroid; n=11 [61%], hypothyroid; and n=38 [72%], euthyroid), followed by toxic goiter (n=13 [20%], hyperthyroid; n=1 [6%], hypothyroid; and n=10 [19%], euthyroid) and thyroiditis (n=6 [33%], hypothyroid; n=4 [8%], euthyroid). In this study, only one patient had congenital hypothyroidism.

In total, 53 (83%) patients in the hyperthyroid group were on anti-thyroid medications. Of them, 11 (17%) received radioactive iodine therapy and anti-thyroid medications. Moreover, 10 (56%) hypothyroid patients were treated with radioactive iodine and required thyroid hormone replacement. Six (33%) patients required thyroid hormone replacement therapy alone. Among them, two (11%) underwent thyroidectomy and required thyroxine replacement therapy after surgery.

In the euthyroid group, 29 (55%) patients achieved euthyroid state with anti-thyroid therapy, and 12 (23%) received radioactive iodine therapy. The patients required thyroxine replacement after radioiodine therapy. Five (9%) patients still required anti-thyroid medications after radioactive iodine therapy, and only one patient did not require any treatment after radioactive iodine therapy. Four (7%) patients did not undergo surgery but were on thyroxine replacement therapy or radioactive iodine before replacement therapy (Table 2).

Table 2: Diagnosis and Treatment According to Groups.

Variables	Hyperthyroid	Hypothyroid	Euthyroid
Diagnosis			
Graves' Disease	51 (80)	11 (61)	38 (72)
Toxic Goiter	13 (20)	1 (6)	10 (19)
Congenital Hypothyroid	-	-	1 (2)
Thyroiditis	-	6 (33)	4 (7)
Treatment			
ATD	53 (83)	-	29 (55)
RAI + ATD	11 (17)	-	5 (9)
Thyroidectomy + Thyroxine	-	2 (11)	2 (4)
RAI + Thyroxine	-	10 (56)	12 (23)
RAI	-	-	1 (2)
Thyroxine	-	6 (33)	4 (7)

ATD: Antithyroid drug; RAI: radioactive iodine. Data describe as n (%)

3.2 Assessment of serum 25(OH)D levels and comparison of serum 25(OH)D, calcium, and phosphate levels among the hyperthyroid, hypothyroid, euthyroid, and control groups

3.2.1 Serum 25(OH)D levels

The serum 25(OH)D level was higher in men than in women in each group (Table 3). More than half of the participants (n=145 [73%]) had vitamin D insufficiency (<50 nmol/L). The euthyroid group had the highest 25(OH) D levels (49.55 [18.57] nmol/L), followed by the hypothyroid group (45.74 [15.17] nmol/L) and the hyperthyroid group (43.6 [20.83] nmol/L). The results were statistically

significant based on the one-way ANOVA (p=0.006, Table 4). However, the control group had the lowest mean serum 25(OH) D level (37.38 [17.21] nmol/L). The post hoc pairwise comparison using the Games-Howell method revealed that the results significantly differed only in the euthyroid and control groups (mean difference [MD]: 12.17; 95% confidence interval [CI]: 2.94, 21.40; p=0.003) (Table 5). Based on the analysis using ANCOVA adjusted for age and gender, the serum 25(OH)D levels remained significantly different (p=0.030, Table 6). The post hoc pairwise comparison using ANCOVA adjusted for age and gender showed that the mean serum 25(OH)D levels differed significantly only between the euthyroid and control groups (MD: 13.94; 95% CI: 5.22, 22.67; p<0.001) (Table 7).

Table 3: Serum 25(OH)D Level In Between Groups, Comparing Male and Female.

Group		N	Mean (SD) (nmol/L)	Mean difference (95% CI)	t-stats (df)	P-value
Hyperthyroid	Male	16	57.35 (28.02)	18.33 (2.88, 33.78)	2.49 (18.22)	0.023
	Female	48	39.02 (15.66)			
Hypothyroid	Male	5	63.48 (16.93)	24.57 (3.84, 45.3)	3.14 (4.53)	0.029
	Female	13	38.91 (6.98)			
Euthyroid	Male	11	63.6 (17.63)	17.73 (6, 29.46)	3.03 (51)	0.004
	Female	42	45.87 (17.16)			
Control	Male	27	44.82 (17.61)	12.85 (4.7, 21)	3.07 (50.39)	0.003
	Female	37	31.96 (14.93)			

Independent sample t-test

Table 4: ANOVA analyses. Comparisons of the mean serum 25 (OH)D, serum calcium and serum phosphate in between groups

Variables (n)	Hyperthyroid *(64)*	Hypothyroid *(18)*	Euthyroid *(53)*	Control *(64)*	F-stat *(df)*	P value
25 (OH) D (nmol/L)	43.6 (20.83)	45.74 (15.17)	49.55 (18.57)	37.38 (17.21)	4.24 (3,195)	0.006
Serum Calcium (mmol/L)	2.20 (0.16)	2.21 (0.11)	2.21 (0.16)	2.32 (0.83)	10.64 (3,195)	<0.001
Serum Phosphate (mmol/L)	1.12 (0.24)	1.05 (0.18)	1.09 (0.25)	1.21 (0.24)	3.21 (3,195)	0.024

Data described as mean (SD)

Table 5: Post hoc pairwise comparison of ANOVA analysis for mean serum 25(OH)D, serum calcium, serum phosphate in between group, using Games- Howell method.

Group comparison	Adj. Mean difference (95% CI)	P-value
Serum 25(OH)D		
Hyperthyroid vs. Hypothyroid	-2.14 (-15.4, 11.12)	>0.95
Hyperthyroid vs. Euthyroid	-5.95 (-15.18, 3.28)	0.524
Hyperthyroid vs. Control	6.22 (-2.57, 15.00)	0.365
Hypothyroid vs. Euthyroid	-3.81 (-17.38, 9.75)	>0.95
Hypothyroid vs. Control	8.35 (-4.91, 21.62)	0.569
Euthyroid vs. Control	12.17 (2.94, 21.40)	**0.003**
Serum Calcium		
Hyperthyroid vs. Hypothyroid	-0.01 (- 0.10,0.08)	0.974
Hyperthyroid vs. Euthyroid	-0.01 (- 0.09,0.71)	0.994
Hyperthyroid vs. Control	-0.12 (- 0.18,- 0.06)	**< 0.001**
Hypothyroid vs. Euthyroid	0.01 (- 0.09,0.10)	0.998
Hypothyroid vs. Control	-0.11 (- 0.19,- 0.03)	0.005
Euthyroid vs. Control	-0.11 (- 0.18,- 0.50)	**< 0.001**
Serum Phosphate		
Hyperthyroid vs. Hypothyroid	0.08 (-0.09, 0.25)	>0.95
Hyperthyroid vs. Euthyroid	0.03 (-0.09, 0.15)	>0.95
Hyperthyroid vs. Control	-0.08 (-0.19, 0.03)	0.335
Hypothyroid vs. Euthyroid	-0.05 (-0.22, 0.13)	>0.95
Hypothyroid vs. Control	-0.16 (-0.33, 0.01)	0.082
Euthyroid vs. Control	-0.11 (-0.23, 0.01)	0.081

Data described as adjusted mean 95% (CI)

Table 6: ANCOVA analysis adjusted for age and gender. Comparisons of the mean serum 25(OH)D, serum calcium and serum phosphate in between groups

Variables	Hyperthyroid (64)	Hypothyroid (18)	Euthyroid (53)	Control (64)	F-stat (df)	P value
25(OH) D (nmol/L)	43.67 (39.16, 58.19)	46.32 (37.8, 54.58)	48.3 (43.26, 53.33)	38.19 (33.64, 42.74)	3.04 (3,194)	0.030

| Serum Calcium (mmol/L) | 2.21 (2.17, 2.24) | 2.24 (2.16, 2.31) | 2.22 (2.18, 2.27) | 2.33 (2.29, 2.36) | 8.322 (3,190) | <0.001 |
| Serum Phosphate (mmol/L) | 1.12 (1.06, 1.18) | 1.04 (0.93, 1.15) | 1.11 (1.04, 1.17) | 1.2 (1.14, 1.25) | 2.64 (3,194) | 0.051 |

Data described as adjusted mean 95% (CI)

Table 7: Post hoc pairwise comparison of ANCOVA analysis for mean serum 25(OH)D, serum calcium and serum phosphate adjusted for age and gender in between groups.

Group comparison	Adj. Mean difference (95% CI)	P-value
Serum 25(OH)D		
Hyperthyroid vs. Hypothyroid	-2.10 (-14.13, 9.92)	>0.95
Hyperthyroid vs. Euthyroid	-5.57 (-14.02, 2.89)	0.484
Hyperthyroid vs. Control	8.38 (0.28, 16.47)	0.038
Hypothyroid vs. Euthyroid	-3.47 (-15.87, 8.94)	>0.95
Hypothyroid vs. Control	10.48 (-1.59, 22.54)	0.130
Euthyroid vs. Control	13.94 (5.22, 22.67)	**<0.001**
Serum Calcium		
Hyperthyroid vs. Hypothyroid	-0.03 (- 0.14, 0.08)	>0.95
Hyperthyroid vs. Euthyroid	-0.02 (- 0.10, 0.06)	>0.95
Hyperthyroid vs. Control	-0.12 (- 0.19, - 0.05)	**< 0.001**
Hypothyroid vs. Euthyroid	0.01 (- 0.10, 0.13)	>0.95
Hypothyroid vs. Control	-0.09 (- 0.20, 0.02)	0.136
Euthyroid vs. Control	-0.10 (- 0.18, - 0.02)	0.004
Serum Phosphate		
Hyperthyroid vs. Hypothyroid	0.08 (-0.09, 0.25)	>0.95
Hyperthyroid vs. Euthyroid	0.01 (-0.1, 0.13)	>0.95
Hyperthyroid vs. Control	-0.08 (-0.19, 0.04)	0.446
Hypothyroid vs. Euthyroid	-0.07 (-0.24, 0.11)	>0.95
Hypothyroid vs. Control	-0.16 (-0.33, 0.01)	0.077
Euthyroid vs. Control	-0.09 (-0.21, 0.03)	0.289

Data described as adjust mean 95% (CI)

3.2.2   Serum calcium levels

The mean serum calcium level was normal in all groups. The level was slightly lower in the thyroid disease group than in the control group. The hyperthyroid group had the lowest mean serum calcium level, followed by the euthyroid and hypothyroid groups. The results were statistically significant (p<0.001). Based on the ANCOVA adjusted for age and gender, the serum calcium levels significantly differed (p<0.001) (Table 6). The post hoc pairwise comparison using ANOVA for serum calcium revealed significant difference between hyperthyroid versus control groups (MD: – 0.12; 95% CI: –0.18, –0.06, p<0.001) and euthyroid versus control group (MD: –0.11, 95% CI: –0.18, –0.50, p<0.001) (Table 5). The post hoc pairwise comparison using ANCOVA for serum calcium (adjusted for age and gender) revealed significant difference only between hyperthyroid versus the control group (MD: – 0.12; 95% CI: –0.19, –0.05, p<0.001) (Table 7).

3.3.3 Serum phosphate level

The mean serum phosphate level was normal in all four groups. Moreover, the control group had a higher mean serum phosphate level than the thyroid disease group (Table 4). Moreover, the hyperthyroid group had a higher serum phosphate level than the euthyroid and hypothyroid groups. The results were statistically significant based on the analysis using one-way ANOVA (p=0.024). However, the post hoc pairwise comparison using the Games-Howell method revealed that the results did not significantly differ. Analysis using ANCOVA adjusted for age and gender revealed that the mean serum phosphate level did not significantly differ, and the results remained insignificant in the post hoc pairwise comparison using ANCOVA (Tables 5, 6, and 7).

3.3 Comparison between Serum Bone Turnover Marker levels among the Hyperthyroid, Hypothyroid, Euthyroid, and Control Groups

3.3.1   Serum CTX) levels

The serum CTX level was used as a bone resorptive marker and the serum P1NP level as a bone formation marker. The hyperthyroid group had the highest mean serum CTX levels, whereas the hypothyroid group had the lowest level. The results were statistically significant (p=0.001, Table 8). The results were statistically significant based on the analysis using ANCOVA adjusted for age and gender   (p=0.002, Table 9). The post hoc pairwise comparisons using the Games-Howell method for ANOVA revealed a significant difference between the hyperthyroid and hypothyroid groups and control group (p<0.001, Table 10). The post hoc pairwise comparison using ANCOVA adjusted for age and gender revealed that the difference in results remained significant between the hyperthyroid and hypothyroid groups (MD: 0.31, 95% CI; 0.07, 0.54; p=0.028). However, the result was not significant between the hypothyroid and control groups. The hyperthyroid and euthyroid groups significantly differed after adjusting for age and gender (MD: –0.19; 95% CI: –0.42, 0.05, p=0.205; Table 11).

3.3.2 Serum P1NP level

The mean serum P1NP level was used as a marker of bone formation. One-way ANOVA revealed that the hyperthyroid group had a higher mean serum P1NP level than the euthyroid and control groups. Further, the hypothyroid group had the lowest mean serum P1NP level. The results were statistically significant (p<0.001, Table 8). Based on the analysis using ANCOVA adjusted for age and gender, the results were significant (p<0.001, Table 9). The post hoc pairwise comparison using the Games-Howell method for ANOVA revealed a significant difference between the hypothyroid and control groups (MD: –26.48 ng/mL, 95% CI: –43.16, –9.8, p <0.001, and between the hyperthyroid and the other groups (p<0.001, Table 10). According to the pairwise comparison using ANCOVA adjusted for age and gender, the results between the hyperthyroid and the other groups were significant. However, no significant difference was noted between the hypothyroid and control groups (Table 11).

Table 8: ANOVA analysis. Comparison between mean serum CTX and serum P1NP in between groups.

Variables (n)		Hyperthyroid *(64)*	Hypothyroid *(18)*	Euthyroid *(53)*	Control *(64)*	F-stat *(df)*	P value
Serum (ng/ml)	CTX	0.48 (0.37)	0.19 (0.12)	0.29 (0.45)	0.39 (0.19)	5.53 (3,195)	**0.001**
Serum (ng/ml)	P1NP	156.54 (114.37)	41.05 (19.3)	73.17 (114.39)	67.53 (34.61)	15.09 (3,195)	**< 0.001**

Data described as mean (SD)

Table 9: ANCOVA analysis adjusted for age and gender. Comparison between mean serum CTX and serum P1NP in between groups.

Variables N	Hyperthyroid *(64)*	Hypothyroid *(18)*	Euthyroid *(53)*	Control *(64)*	F-stat *(df)*	P value
Serum CTX (ng/ml)	0.48 (0.4,0.56)	0.18 (0.03, 0.33)	0.3 (0.21,0.39)	0.38 (0.29, 0.46)	5.25 (3,193)	**0.002**
Serum P1NP (ng/ml)	156.32 (134.19,178.45)	39.35 (- 2.43, 81.12)	76.86 (52.17, 101.55)	65.18 (42.89,87.48)	15.33 (3,194)	**< 0.001**

Data described as adjusted mean 95% (CI)

Table 10: Pairwise comparison of mean bone turnover markers in between groups (ANOVA). Multiple comparisons using Games-Howell method.

Group comparison	Adj. Mean difference (95% CI)	P-value
Serum CTX		
Hyperthyroid vs. Hypothyroid	0.3 (0.16, 0.44)	**<0.001**
Hyperthyroid vs. Euthyroid	0.2 (0, 0.4)	0.054
Hyperthyroid vs. Control	0.1 (-0.04, 0.23)	0.236
Hypothyroid vs. Euthyroid	-0.1 (-0.28, 0.08)	0.481
Hypothyroid vs. Control	-0.2 (-0.3, -0.1)	**<0.001**
Euthyroid vs. Control	-0.1 (-0.28, 0.07)	0.426
Serum P1NP		
Hyperthyroid vs. Hypothyroid	115.48 (76.04, 154.92)	**<0.001**
Hyperthyroid vs. Euthyroid	83.37 (27.95, 138.78)	0.001
Hyperthyroid vs. Control	89.01 (49.75, 128.26)	**<0.001**

Hypothyroid vs. Euthyroid	-32.12 (-75.35, 11.11)	0.213
Hypothyroid vs. Control	-26.48 (-43.16, -9.8)	**0.001**
Euthyroid vs. Control	5.64 (-37.43, 48.71)	0.986

Data described as adjust mean 95% (CI)

Table 11: Post hoc pairwise comparison between mean of bone turnover markers adjusted for age and gender in between groups (ANCOVA)

Group comparison	Adj. Mean difference (95% CI)	P-value
Serum CTX		
Hyperthyroid vs. Hypothyroid	0.31 (0.07, 0.54)	**0.004**
Hyperthyroid vs. Euthyroid	0.18 (0.01, 0.34)	0.028
Hyperthyroid vs. Control	0.12 (-0.04, 0.28)	0.280
Hypothyroid vs. Euthyroid	-0.13 (-0.37, 0.11)	0.926
Hypothyroid vs. Control	-0.19 (-0.42, 0.05)	0.205
Euthyroid vs. Control	-0.06 (-0.23, 0.11)	>0.95
Serum P1NP		
Hyperthyroid vs. Hypothyroid	117.02 (52.97, 181.08)	**<0.001**
Hyperthyroid vs. Euthyroid	79.38 (34.35, 124.41)	**<0.001**
Hyperthyroid vs. Control	91.38 (48.29, 134.48)	**<0.001**
Hypothyroid vs. Euthyroid	-37.64 (-103.73, 28.45)	0.784
Hypothyroid vs. Control	-25.64 (-89.9, 38.62)	>0.95
Euthyroid vs. Control	12 (-34.47, 58.47)	>0.95

Data described as adjust mean 95% (CI)

3.4 Comparison of BMD among the Hyperthyroid, Hypothyroid, Euthyroid, and Control Groups

3.4.1   Z-score/bone density (g/cm^2)

Of 199 patients, only 186 underwent BMD measurement. Moreover, 13 participants did not attend the BMD appointment. Bone density (g/cm^2) and z-score (bone density compared according to similar age and sex) of the left hip and spine were analyzed. Based on the analysis of z-score for left hip and spine using one-way ANOVA, the thyroid group was found to have lower hip z-scores than the control group (0.39 [1.03]). However, the results were not statistically significant (p=0.164, Table 12). The post hoc pairwise comparison using the Bonferroni method for ANOVA revealed no significant difference among the hyperthyroid, hypothyroid, euthyroid, and control groups (Table 13). Based on the analysis of covariates and post hoc pairwise comparison with ANCOVA adjusted for age and gender, the results were not statistically significant among the groups (Tables 14 and 15).

ANOVA for bone density (g/cm^2) of the left hip and spine revealed that the control and euthyroid groups had a higher bone density than the hypothyroid and hyperthyroid groups. The results for the left hip bone density was statistically significant (p=0.002, Table 12). The analysis using ANCOVA adjusted for age and gender revealed that the difference in the left hip bone density remained statistically significant (p=0.011, Table 14). Based on the post hoc pairwise comparison

using ANOVA and ANCOVA, the left hip bone density between the hyperthyroid and control groups differed significantly (Tables 13 and 15).

1

Table 12: ANOVA analysis. Comparison the mean of Z score for Hip and Spine, and bone density (g/cm2) for Hip and Spine in between groups

Variables(*n*)	Hyperthyroid (*57*)	Hypothyroid (*15*)	Euthyroid (*50*)	Control (*64*)	F-stat (df)	P value
Hip Z score	-0.02	0.25	0.27 (1.15)	0.39 (1.03)	1.72 (3,182)	0.164
	(0.83)	(0.95)				
Spine Z score	-0.29	-0.65	-0.18 (1.09)	-0.48 (1.03)	1.3 (3,182)	0.275
	(0.93)	(0.79)				
Hip g/cm²	0.86	0.93	0.91 (0.15)	0.95 (0.13)	5.12 (3,182)	**0.002**
	(0.11)	(0.14)				
Spine g/cm²	0.97	0.97	1.01 (0.13)	1.00 (0.13)	1.197 (3,182)	0.312
	(0.09)	(0.09)				

Data described as mean (SD)

Table 13: Pairwise comparison of Z score and bone density (g/cm²) of hip and spine among groups (ANOVA). Multiple comparisons using Bonferroni method

Group comparison	Adj. Mean difference (95% CI)	P-value
Hip (Z Score)		
Hyperthyroid vs. Hypothyroid	-0.27 (-1.05, 0.51)	>0.95
Hyperthyroid vs. Euthyroid	-0.29 (-0.81, 0.23)	0.845
Hyperthyroid vs. Control	-0.41 (-0.9, 0.08)	0.162
Hypothyroid vs. Euthyroid	-0.02 (-0.81, 0.77)	>0.95
Hypothyroid vs. Control	-0.14 (-0.91, 0.63)	>0.95
Euthyroid vs. Control	-0.12 (-0.63, 0.39)	>0.95
Spine (Z Score)		
Hyperthyroid vs. Hypothyroid	0.36 (-0.42, 1.13)	>0.95
Hyperthyroid vs. Euthyroid	-0.11 (-0.62, 0.41)	>0.95
Hyperthyroid vs. Control	0.19 (-0.3, 0.67)	>0.95
Hypothyroid vs. Euthyroid	-0.46 (-1.25, 0.32)	0.713
Hypothyroid vs. Control	-0.17 (-0.94, 0.6)	>0.95
Euthyroid vs. Control	0.29 (-0.21, 0.8)	0.743
Hip (g/cm²)		
Hyperthyroid vs. Hypothyroid	0.08 (- 0.18, 0.03)	0.248
Hyperthyroid vs. Euthyroid	-0.05 (- 0.12, 0.01)	0.174
Hyperthyroid vs. Control	-0.09 (-0.15, -0.03)	**<0.001**

Hypothyroid vs. Euthyroid	0.02 (-0.09, 0.14)	0.944
Hypothyroid vs. Control	-0.02 (-0.13, 0.10)	0.974
Euthyroid vs. Control	- 0.04 (-0.11, 0.03)	0.445
Spine (g/cm²⁾		
Hyperthyroid vs. Hypothyroid	-0.01(-0.07, 0.07)	>0.95
Hyperthyroid vs. Euthyroid	-0.04(-0.10, 0.02)	0.337
Hyperthyroid vs. Control	-0.03 (-0.08,0.02)	0.463
Hypothyroid vs. Euthyroid	-0.04 (-0.012, 0.04)	0.607
Hypothyroid vs. Control	-0.03 (-0.11, 0.55)	0.731
Euthyroid vs. Control	0.01 (-0.06,0.07)	0.992

Data described as adjust mean 95% (CI)

Table 14: ANCOVA analysis adjusts for age and gender. Comparisons of the Z score mean for hip and spine and bone density (g/cm²) for hip and spine in between groups.

Variables (n)	Hyperthyroid (57)	Hypothyroid (15)	Euthyroid (50)	Control (64)	F-stat (df)	P value
Hip Z score	0.02 (-0.26, 0.3)	0.28 (-0.24, 0.79)	0.24 (-0.06, 0.55)	0.43 (0.18, 0.68)	1.62 (3,180)	0.187
Spine Z score	-0.26 (-0.54,0.02)	-0.62 (-1.14, -0.11)	-0.17 (-0.47, 0.13)	-0.46 (-0.71, -0.21)	1.24 (3,180)	0.588
Hip g/cm²	0.877 (0.84,0.92)	0.95 (0.88, 1.02)	0.91 (0.88,0.96)	0.96 (0.93, 0.99)	3.81 (3,177)	**0.011**
Spine g/cm²	0.98 (0.94,1.02)	0.98(0.91,1.05)	1.01 (0.97, 1.05)	1.01 (0.98, 1.04)	0.60 (3,177)	0.617

Data described as Adj. Mean (95% CI).

Table 15: Pairwise comparison of Z score and bone density (g/cm^2) of hip and spine among groups adjust for age and gender (ANCOVA)

Group comparison	Adj. Mean difference (95% CI)	P-value
Hip (Z Score)		
Hyperthyroid vs. Hypothyroid	-0.26 (-1.03, 0.51)	>0.95
Hyperthyroid vs. Euthyroid	-0.23 (-0.75, 0.30)	0.845
Hyperthyroid vs. Control	-0.41 (-0.90, 0.09)	0.178
Hypothyroid vs. Euthyroid	-0.03 (-0.76, 0.82)	>0.95
Hypothyroid vs. Control	-0.15 (-0.92, 0.62)	>0.95
Euthyroid vs. Control	-0.18 (-0.71, 0.35)	>0.95
Spine (Z Score)		
Hyperthyroid vs. Hypothyroid	0.36 (-0.42, 1.14)	>0.95
Hyperthyroid vs. Euthyroid	-0.09 (-0.62, 0.44)	>0.95
Hyperthyroid vs. Control	0.20 (-0.30, 0.70)	>0.95
Hypothyroid vs. Euthyroid	-0.45 (-1.25, 0.34)	0.777
Hypothyroid vs. Control	-0.16 (-0.94, 0.61)	>0.95
Euthyroid vs. Control	0.29 (-0.24, 0.82)	0.849
Hip (g/cm^2)		
Hyperthyroid vs. Hypothyroid	-0.07 (- 0.15, 0.01)	0.521
Hyperthyroid vs. Euthyroid	-0.04 (- 0.12, 0.04)	>0.95
Hyperthyroid vs. Control	-0.09 (-0.14, -0.03)	**<0.001**
Hypothyroid vs. Euthyroid	0.04 (-0.88, 0.15)	>0.95
Hypothyroid vs. Control	-0.01 (-0.12, 0.10)	>0.95
Euthyroid vs. Control	-0.05 (-0.12, 0.02)	0.481
Spine (g/cm^2)		
Hyperthyroid vs. Hypothyroid	-0.01(-0.11, 1.00)	>0.95
Hyperthyroid vs. Euthyroid	-0.03(-0.10, 0.05)	>0.95
Hyperthyroid vs. Control	-0.03 (-0.10,0.04)	>0.95
Hypothyroid vs. Euthyroid	-0.02 (-0.13, 0.08)	>0.95
Hypothyroid vs. Control	-0.02 (-0.12, 0.08)	>0.95
Euthyroid vs. Control	0.01 (-0.07,0.07)	>0.95

Data described as adjust mean 95% (CI)

4. Discussion

Thyroid diseases have widespread systemic manifestations. That is, they affect the bone. However, thyrotoxicosis and hypothyroidism have a different impact on bone metabolism. Hyperthyroidism is an important cause of secondary osteoporosis and is associated with significant bone loss at the hip, forearm, and lumbar spine [16]. Thyroid-stimulating hormones (TSH) directly affect bone remodeling via the TSH receptor, which is found on osteoblasts and osteoclast precursor cells. [17]

Vitamin D plays a pivotal role in the development and maintenance of a healthy skeleton. Moreover, it plays a critical function in calcium and phosphorus metabolism and ensures an adequate level of these minerals for bone mineralization [18]. The serum 25(OH)D concentration is an indicator of vitamin D status. It reflects vitamin D level from endogenous production and food or supplements [19], and it has a relatively long circulating half-life (15 days) than 1,25(OH)2D (15 h) [20].

In this study, the 25(OH)D levels range from 12.26 to 111.22 nmol/L. However, the mean serum 25(OH)D levels in patients with thyroid diseases and healthy controls were below the adequate level (<50 nmol/L). These findings might be attributed to the fact that studied subjects do not consume vitamin D-fortified food or vitamin D supplements. Even if vitamin D is consumed, it may not be enough as per the value recommended by the Endocrine Society Clinical Practice Guideline.

1   That is, adults are required to intake 1500–2000 IU of vitamin D [21]. The healthy controls and patients with thyroid disease differed in terms of prolonged exposure to sunlight while going for treatment. In contrast, the healthy control group included hospital staff who had less exposure to sunlight due to working their hours that start from 8 am to 5 pm and they stay outside the building for an extremely limited period within the day. In addition, only few studies assessed 25(OH)D levels different study cohorts in Malaysia [22-25]. A systematic review of vitamin D status in populations worldwide conducted in 2013 revealed that 37.3% of the participants had a vitamin D level <50 nmol/L, and 6.7% had values <25 nmol/L. [26] The mean vitamin D levels among individuals living in countries in the South East Asia region such as Indonesia and Malaysia are 38.7 and 67.6 nmol/L, respectively [27,28]. Even though both are tropical countries similar to Malaysia, the result significantly differed.

Factors such as dietary intake and daily duration of sun exposure, seasonal variation, physical activity, rural/urban area, skin pigmentation, sun-protective behavior can influence serum vitamin D level. In Malaysia, Nurbazlin et al. (2013) reported that the 25(OH)D concentration in women living in rural area was significantly higher than that in women living in urban area [24]. Seasonal variations affect serum 25(OH)D concentrations in patients with Graves' disease. This result is supported by a study showing that that serum 25(OH)D concentrations were high during summer and low during winter [29]. Another research showed a slight decrease in the mean serum vitamin D concentration during winter compared with during summer [30]. The serum 25(OH)D concentration was higher in men than in women. Yamashita et al. (2005) reported the occurrence of hypovitaminosis D in 40% of women and 18% of men with Graves' disease in Japan [29]. Meanwhile, Mackay AM et al. (2013) showed that the serum 25(OH)D level was lower in hypothyroid women than in hypothyroid men [31]. In Kuala Lumpur, Malaysia, a study on adults revealed that approximately 41% of men and 87% of women had insufficient of vitamin D levels (<50 nmol/L) [8]. However, there is no clear evidence on differences in terms of gender. However, a large study on obese adult women revealed that the possible pathophysiology is the deposition of vitamin D in the subcutaneous fat, which is released when there is a decreased cutaneous production [32]. Nonetheless, in our study, the mean BMI among the participant commonly ranges from 23.69 to 25.75 kg/m². This finding could not explain this condition. One cause is that men are more exposed to sunlight due to their clothing. By contrast, women preferred clothing that covers the entire body regardless of weather conditions. Other reasons include less sun exposure, few outdoor activities, and limited body area exposure in women than in men.

This study also found that the mean serum 25(OH)D concentration was higher in patients with thyroid disease than in healthy controls. This finding was not in accordance with those of other studies conducted in different countries. For example, Wang, Jiying et al. (2015) revealed that thyrotoxic patients had a significantly lower plasma 25(OH) vitamin D level than controls [33]. Moreover, Dhanwal et al. (2010) had examined the vitamin D level in premenopausal hyperthyroid patients. Results showed that the 25(OD)D levels of 26.6% of patients with hyperthyroidism was l<25.0 nmol/L, and 73.4% had sufficient vitamin D levels, with a mean (SD) of 46.0 (13.5) nmol/L[5]. Similarly, Mackawy AM et al. (2013) had reported that the serum 25(OH)D level was significantly lower in hypothyroid patients than in controls [31]. However, several studies did not show a correlation between low vitamin D level and autoimmune thyroid diseases or Hashimoto thyroiditis. Goswami et al. reported that no correlation between vitamin D deficiency and anti-TPO positivity in 642 students, teachers, and staff in India. However, only a poor reverse association was noted between serum 25(Oh)D levels and anti-TPO (r=−0.08; p=0.04). [34] Although there was a higher number of men than women in this study, the results of the current study were contrasting with those of other studies. These discrepancies might be attributed to the differences in the patient selection process, varying background characteristics, and residents of patients with thyroid diseases

All groups had normal mean serum total calcium and phosphate levels. However, the mean calcium levels were higher in the control group than in the thyroid disease group, and the phosphate level was higher in the control group than in the thyroid disease group. However, the result was not significantly different. The results of previous studies were heterogeneous. In the study of Dhanwal *et*

al. (2010) conducted in India, hyperthyroid patients only presented with slightly high serum calcium levels. However, the difference was not significant, and the serum phosphate level was normal [5]. Anuj Modi et al. (2018) showed that the serum calcium and phosphate levels were significantly higher in the hyperthyroid group than in the control group [35]. Moreover, Sridevi et al. (2016) reported that the hypothyroid group had lower calcium levels and higher serum phosphate levels than the control group [36].

Overt hyperthyroidism leads to the acceleration of bone turnover and loss of bone mineral density by 10%–20% mainly in the cortical bone [37–39]. The cycle of bone remodeling is shortened by almost 50% (from 200 to 113 days), which disturbs the proportion of bone formation and bone resorption [3]. The phase of bone formation is reduced by 2/3, which results in the loss of <10% of mineralized bone in one cycle. [40] Hypothyroidism causes hypometabolism in general. Bone formation mechanisms decelerate in 40% of bone resorption processes. [3]

The negative impact on bone equilibrium is initially expressed as an increase or decrease in bone turnover markers. Our findings showed that the bone turnover markers are higher in hyperthyroid patients and lower in hypothyroid patients. The euthyroid and control groups had a similar level of bone turnover markers. The results indicate that hyperthyroid patients had accelerated bone resorption and formation, whereas hypothyroid patients had reduced bone turnover. Restoration to euthyroid level after treatment for hyperthyroid and hypothyroid can improve bone turnover.

Few studies showed persistent suppression of TSH levels, which result in a continuous increase in bone turnover even after the normalization of serum FT3 and FT4 levels [41,42]. As a higher bone turn over resulted in a continuous accelerated bone loss [43-46], TSH suppression might be a risk factor for secondary osteoporosis in patients with Graves' disease. Therefore, to normalize bone metabolism with anti-thyroid drug in patients with Graves' disease, the serum TSH should be maintained within the reference range. The result of the current study on bone turnover was consistent with that of previous studies [41,43].

In the current study, bone densitometry showed that the z-score of the left hip and spine and bone density (g/cm^2) of the spine were not significant. Only bone densities (g/cm^2) of the left hip in hyperthyroid patients were significant. These findings differed from other studies. Rosario et al. (2008) showed a significant increase in the serum markers of bone formation and resorption in <65-year-old premenopausal women with subclinical hyperthyroid. In premenopausal patients, the bone mineral density in the femoral neck but not in the lumbar spine was lower. In contrast, other studies showed that postmenopausal patients had low BMD at both sites [47]. Tauchmanova et al. (2004) examined 60 patients with endogenous subclinical hyperthyroidism due to multinodular goiter. The study included 30 premenopausal and 30 early postmenopausal women. Results showed a significant decrease in femoral BMD both in pre- and postmenopausal women compared with controls. However, postmenopausal women had a higher femoral BMD. [48] Our study the study of Tuchendler et al., (2013), which showed newly diagnosed hypothyroidism among premenopausal women (average age: 33.37 [10.83] years) did not influence bone density. [49] Similarly, Hanna et al. (1998) showed no difference in terms of bone mineral density in patients receiving replacement doses of thyroxine irrespective of the etiology of hypothyroidism. [50] A demographic analyses of 4473 autoimmune hypothyroid participants have shown that the incidence of fractures increased during diagnosis. [51]. However, the discovery of a possible pre-diagnosis correlation (i.e., before beginning T4 therapy) is contrast to the role of excess thyroid hormones based on these results. However, there are still limited data on the association between the risk of fracture and T4 treatment, even at doses appropriate for TSH serum suppression. Moreover, precaution must be observed in high-risk groups, particularly postmenopausal women and, possibly, men. [52] However, reduced bone mineral density associated with hyperthyroidism may be restored, maintained, and, in some cases, improved while on long-term thyroxine replacement after radioiodine therapy. [50]

The current study had several limitations. First, the characteristic of a cross-sectional study does not reflect results over time. Moreover, the sample size was small, particularly in the hypothyroid group. Most participants were Malay. Hence, this does not extrapolate the condition of other

populations. The assessment of thyroid functions is based on a single assessment. Moreover, in this study, the cofounders of serum vitamin D level have not been addressed.

We recommend a prospective longitudinal study with a longer duration. Moreover, a more significant population should be included, and the study must be conducted at multiple centers. Assessment of average thyroid function better represents the stage of thyroid function within the last 6 months. Confounders correlated to vitamin D measurements such as duration of daily sun exposure and dietary intake assessment will provide more value to vitamin D status in patients with thyroid disease and control groups.

5. Conclusions

In this study, serum 25(OH)D levels were not correlated with thyroid diseases. All groups had normal serum calcium and phosphate levels. The serum bone turnover marker levels were significantly high in the hyperthyroid group and low in the hypothyroid group. There was no difference in terms of z-score bone density among all groups. However, the bone density of the left hip was significantly low in the hyperthyroid group.

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