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Association between 25-hydroxy Vitamin D_3 levels and thyroiditis staged by using ultrasonography

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ABSTRACT

Vitamin D deficiency has been linked to a higher prevalence of thyroid gland impairment. The association between 25-hydroxy Vitamin D_3 concentrations and sonographic changes of thyroiditis was investigated. A total of 55 patients were divided into 3 groups according to the thyroid ultrasonography (US) findings: Group 1: control cases (n=17); Group 2: intermediate (early) cases (n=14); and Group 3: active and late-stage thyroiditis cases (n=24). Serum 25-hydroxy Vitamin D concentrations, thyroid hormones (free T3, free T4, TSH) and antibody levels (TPO Ab, Tg Ab) were assessed in different stages of thyroiditis. The Vitamin D concentrations of Group 1 (28.54 ± 20.43 ng/dL) were significantly higher than those of Group 2 (14.48 ± 5.87 ng/mL) and Group 3 (14.3 ± 9.2 ng/dL) ($p=0.025$ and $p=0.004$, respectively). Tg Ab and TPO Ab significantly increased in Group 3 compared to Groups 1 and 2 ($p<0.001$, $p<0.001$, respectively). 25-hydroxy Vitamin D_3 deficiency was associated with thyroid gland morphological changes detected by ultrasonography.

Keywords: 25-OH Vitamin D_3, autoimmune thyroiditis, anti-Tg, anti-TPO, sonography

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INTRODUCTION

Vitamin D is a well-known autoimmune system regulator. Vitamin D deficiency has been linked to a higher prevalence of thyroid autoimmunity in certain populations, including children, adolescents, and obese individuals. Moreover, Vitamin D supplementation has shown promise in reducing antithyroid antibody levels, improving thyroid function[1-3]. However, while some papers publish evidence that Vitamin D is an important factor in developing autoimmune thyroiditis[4-9], some papers report that their data do not provide any relationship[10,11].

Conventional method of diagnosing autoimmun thyroiditis (AT) is detecting anti-thyroglubulin (anti-Tg) and anti-thyroperoxidase (anti-TPO) high in serum[12]. During the development of autoimmune thyroiditis, structural changes occur in the gland. Hashimato Thyroiditis (HT) is characterized pathologically by lymphocytic infiltration of the interstitium, mainly lymphocytes with some plasma cells and macrophages[12,13]. The lymphoid tissue is distributed within and around the lobules and often exhibits large follicles with prominent germinal centers[12,14]. The inflammatory process also results in oxyphilic changes in follicular epithelial cells, parenchymal atrophy of thyroid tissue, and varying degrees of fibrosis, imparting a firm consistency to the thyroid[15]. These changes in the gland can be observed by ultrasonography. Thyroid ultrasonographic evaluation of patients with HT generally reveals diffuse enlargement of the gland, a heterogeneous background, and a general decrease in echogenicity. Other sonographic findings of HT often include hypervascularity and the presence of hypoechoic micronodules with an echogenic rim[16,17]. Some authors advocate the use of ultrasonography in cases of AT[18,19] because it can provide information about the level of inflammatory activity[20] and thyroiditis severity[21]. The histopathological findings and sonographic features in different stages of AT have been reported and used in clinical practice[22].

In this study, the association between thyroiditis and 25-hydroxy Vitamin D_3 levels was evaluated during different stages of thyroiditis based on morphological changes in the thyroid gland by using ultrasonography.

METHODOLOGY

The study groups

All patients (n=253) who were referred to our study radiologist for thyroid gland ultrasound (US) between June 2013 and February 2014 were included in the data. Among the patients, pregnant and breastfeeding women, pediatric patients, and patients who had undergone surgery and those with known malignancy, short neck, marked goiter, and thyroid nodules were excluded.

After exclusion, the study was conducted with 55 cases (15 male, 40 female). All patients underwent physical examination, thyroid hormones (free T_3, free T_4 and TSH) and 25-hydroxy Vitamin D_3 were analyzed, and thyroid sonography evaluation was performed. This study was approved by the Medipol University Ethics Committee.

Sonographic evaluation of thyroid gland

In this study, thyroiditis staging by ultrasonography was performed and reported as described by Ormeci et al.[22]. Briefly, normal thyroid parenchyma has a homogeneous medium-high level of echogenicity compared to periglandular muscles, diffuse tissue enlargement, parenchymal hypoechogenicity and coarsening. During US examination, the radiologist reported the thyroid gland sonographic findings as the echo texture (homogeneous or heterogeneous), echogenicity (hypo- or hyperechoic), contouring (regular or irregular), nodulation or pseudonodulation, and vascularization of the thyroid gland (decreased, normal, or increased) (Table 1)[22].

Table 1. Sonographic features of the groups[22]

Sonographic criteria	Group 1 Normal thyroid	Group 2 Early/indeterminate thyroiditis	Group 3 Chronic thyroiditis
Volume	Normal	Increased, normal	Decreased
Echotexture	Homogeneous	Homogeneous, slightly heterogenous	Heterogeneous
Echogenicity (compare to the muscles)	Hyperechoic	Hyperechoic, slightly hypoechoic	Hypoechoic
Contouring	Smooth	Smooth	Irregular
Nodulation/ pseudonodulation	No	No, slightly nodular	Pseudonodulation
Vascularization	Normal	Normal, increased	Increased, normal, decreased

Patients were grouped by thyroid gland sonographic features according to the properties described above, and the patients were divided into 3 groups according to sonography results as described in Table 1.

Group 1 (control cases; Figure 1-a) consisted of cases with normal thyroid gland ultrasonography, clinical, and laboratory findings. Group 2 (intermediate/earlystage cases; Figure 1-b) consisted of cases with minimal parenchymal hypoechogenicity and suspected heterogeneity but were still clinically suspected of autoimmune thyroiditis. Group 3 (active and advanced-late-stage thyroiditis patients; Figure 1-c and 1-d) consisted of patients in whom the parenchyma was heterogeneous/hypoechogenic in appearance and who had interlobular septa, irregular contours, pseudonodules, and glands of reduced size[22]. Ultrasound evaluation of thyroid gland was performed by a single radiologist who was blind to the thyroid hormones data. A Doppler device (LOGIQ P6 Pro, GE Healthcare GmBH, Germany) fitted with a linear 11 MHz probe was used.

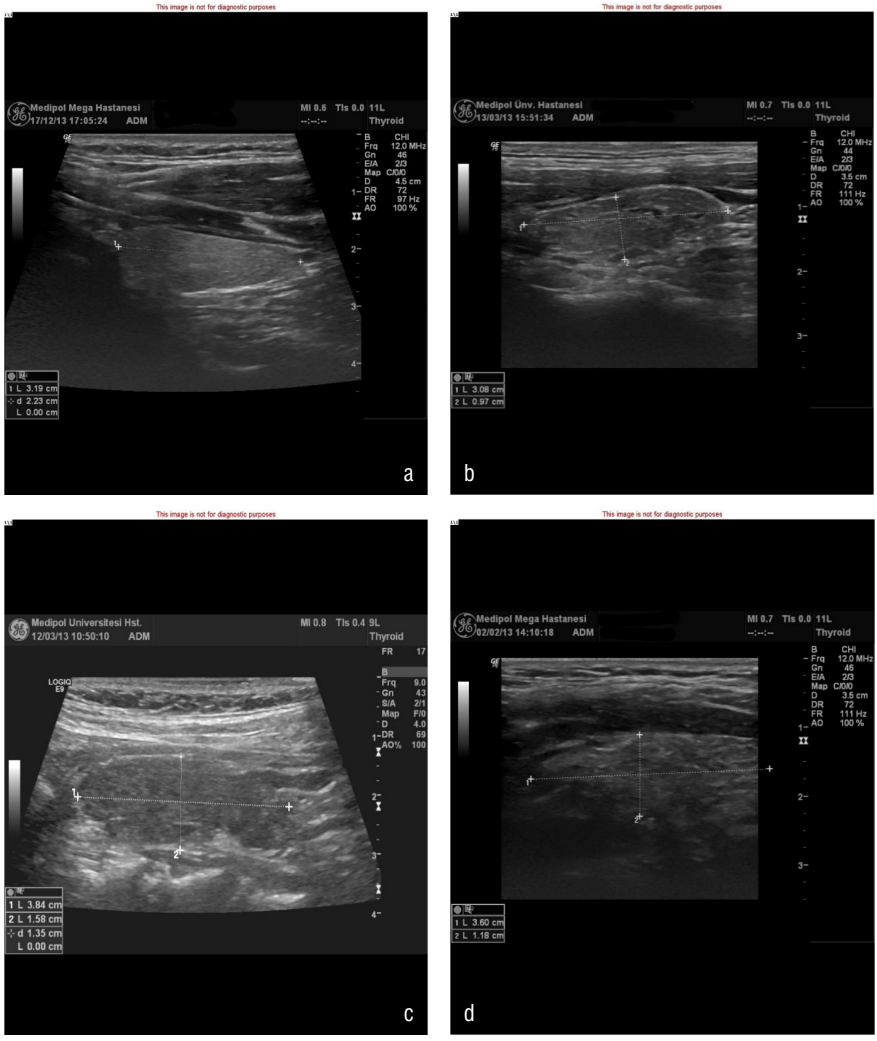

Figure 1. (a) Group 1 (control cases) normal thyroid gland ultrasonography, **(b)** Group 2 (intermediate/early-stage cases); minimal parenchymal hypoechogenicity and suspected heterogeneity, **(c)** Group 3 (active and advanced-late stage) parenchyma was heterogeneous/hypoechogenic, **(d)** Group 3 (chronic stage) with parenchyma was heterogeneous/hypoechogenic.

Hormone and 25-hydroxy Vitamin D_3 analysis

Free triiodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH), anti-thyroid antibody (TPO Ab), anti-thyroglobulin (Tg Ab) and 25-hydroxy Vitamin D tests were performed by using electrochemiluminescence kits and an automated analyzer (COBAS e 6000 ROCHE). Serum samples from all patients were kept at -20°C until the end of data collection.

We used a double-blind approach throughout the data collection period. Both the clinical biochemist and radiologist were blinded to the patients' clinical and study data throughout the data collection period.

Statistical analysis

SPSS for Windows was used for statistical analysis. The data are presented as the mean ± standard deviation (SD), median, frequency, ratio, and minimum to maximum. According to the Kolmogorov-Smirnov test, the distributions of free triiodothyronine (fT3), free thyroxine (fT4), thyroid-stimulating hormone (TSH), anti-thyroid antibody (TPO Ab), anti-thyroglobulin (Tg Ab) and 25-hydroxy Vitamin D_3 test data were nonuniform ($p<0.05$). The Kruskal-Wallis test was used to compare three or more groups, and the Mann-Whitney U test was used to identify the group responsible for any observed difference.

RESULTS and DISCUSSION

Characteristics of the study groups

A total of 55 patients, consisting of 40 (72.7%) female and 15 (27.3%) male patients, were included in the study after the exclusion criteria. The mean age of all patients was 37.49 ± 11.76 (range between 18-64) years (Table 2).

As in the current study, thyroiditis was staged according to sonographic evaluation as described by Ormeci et al. in Table 1, and patients were grouped into 3 stages[22]. Subjects with no glandular disruption in the thyroid gland were called the group-1 control group and consisted of 17 patients (7 Female/10 Male). Early- and intermediate-stage thyroiditis cases in group 2 consisted of 14 (10 Female/4 Male) individuals. In group 3, chronic and late-stage thyroiditis cases consisted of 24 (23 Female/1 Male) people. Among the groups, we observed that the number of women increased significantly as the thyroiditis stage progressed. There was no significant difference between the groups in terms of age distribution ($p=0.607$; Kruskal-Wallis test) (Table 2).

Table 2. Characteristics of the subjects and thyroid hormones, anti-thyroid antibodies and 25-hydroxy Vitamin D_3

Thyroiditis staging according to sonography evaluation	N	Age years Mean ± SD Median min-max	Sex	free T3 (pg/mL) Mean ± SD Median min-max	free T4 (ng/dL) Mean ± SD Median min-max	TSH (μIU/mL) Mean ± SD Median min-max	anti-Tg Ab (IU/mL) Mean ± SD Median min-max	anti-TPO Ab (IU/mL) Mean ± SD Median min-max	25-OH Vit D3 (ng/mL) Mean ± SD Median min-max
Group 1 Control Cases no confirmed thyroiditis	17	35,6 ± 11,5 36,5 18-64	7F 10M	2,99 ± 0,42 (3,00) 2,14-3,60	1,23 ± 0,25 (1,17) 0,92-1,93	2,11 ± 1,68 (1,73) 0,45-9,59	18,97 ± 10,87 (17,00) 10,00-58,00	10,80 ± 13,37 (5,87) 5,00-63,50	28,54 ± 20,43 (22,4) 6,39-70,00
Group 2 Early/ intermediate-stage thyroiditis cases	14	38,4 ± 10,3 (36,0) 20-60	10F 4M	3,16 ± 0,36 (3,26) 2,51-3,89	1,18 ± 0,42 (1,24) 0,1-2,19	4,31 ± 11,64 (1,90) 0,016-65,0	21,82 ± 9,63 (20,00) 10,00-42,28	24,92 ± 55,97 (7,07) 5,00-238	14,48 ± 5,87# (15,00) 4,91-23,62
Group 3 Active and late-stage thyroiditis cases	24	37,9 ± 12,5 (34,0) 19-63	23F 1M	3,71 ± 2,95 (2,83) 2,15-18,66	1,43 ± 1,69 (1,08) 0,30-13,0	8,68 ± 19,04** (3,41) 0,005-100	275 ± 300** (174,5) 14-1262	306 ± 423** (266) 5-2550	14,3 ± 9,2* (12,66) 3,16-34,79
p-value Kruskal-Wallis Test		0,607	-	0,474	0,214	0,006	0,001	0,001	0,028

*p<0,05 Group 2 compared to Control group by using Mann-Whitney U Test.
** p<0,01 Group 3 compared to Control group by using Mann-Whitney U Test.
# p<0,05 Group 2 compared to Control group by using Mann-Whitney U Test.
F stands for female
M stands for male

Relationship between thyroid hormones, anti-thyroid antibodies and 25-hydroxy Vitamin D_3

There was no significant difference between free T_3 and free T_4 hormones among the 3 groups. However, TSH was significantly elevated in group 3, which included patients with chronic active thyroiditis (p<0.01; Mann-Whitney U Test). This shows us that the TSH level changes significantly and noticeably only in the active and chronic stages (group 3), and the thyroid gland has to work harder. When we examined the anti-Tg Ab and anti-TPO Ab levels that we used in the diagnosis of autoimmune thyroiditis, we observed that they increased significantly in the chronic and active thyroiditis stages (group 3) compared to group 1 and group 2 (p<0.01, Mann-Whitney U test).

When we analyzed 25-hydroxy Vitamin D_3 levels, they were 28.54 ± 20.43 ng/dL in people with normal thyroiditis (group 1), 14.48 ± 5.87 ng/mL in group 2 and 14.3 ± 9.2 ng/mL in group 3. It was determined to be 14.3 ± 9.2 ng/mL. The vitamin D results of patients in both group 2 and group 3 were found to be significantly lower than those in group 1 ($p<0.05$, Mann-Whitney U test). Even before anti-thyroid antibodies are produced in the body and provide evidence about the nature of thyroiditis disease, patients have already developed Vitamin D deficiency.

In the current study, the association between autoimmune thyroiditis and 25-hydroxy Vitamin D_3 levels was associated during different stages of thyroiditis based on morphological changes in the thyroid gland by using ultrasonography. According to the results, 25-hydroxy Vitamin D_3 levels were found significantly lower in patients who develop early signs of morphological changes in thyroid gland which were evaluated by ultrasound examination. When anti-thyroid antibodies (anti-Tg and anti-TPO) were positive in the serum, thyroid gland showed severe destructions and patients had already developed 25-hydroxy Vitamin D_3 deficiency.

In the literature, there were conflicting results regarding whether 25-hydroxy Vitamin D_3 levels play an important role in the development of autoimmune thyroiditis. While some papers gave evidence that 25-hydroxy Vitamin D_3 was an important factor in autoimmune thyroiditis[23, 24] and some papers reported their data do not provide any relationship[25].

In our opinion, the design of grouping patients in each study was based on different criteria, which is one of the main reasons for conflicting results in the literature. The diagnosis of AT is conventionally based on clinical findings and laboratory tests, such as elevated thyroglobulin antibody (Tg Ab) and thyroid peroxidase antibody (TPO Ab). However, antibodies can be negative in cases of thyroiditis, as evidenced by a histological examination[11]. Some authors advocate the use of ultrasonography in cases of AT[15,16], where it was found to be useless by other authors[17]. The combination of US with clinical and serological assessments significantly improves sensitivity and specificity for diagnosing AT[18,19].

It has been demonstrated that 25-hydroxy Vitamin D_3 plays an important role in the immune system. The possible effects of low 25-hydroxy Vitamin D in the early stages of thyroiditis D'Aurizio et al. have discussed in depth[26]. Calapkulu et al. also found decreased levels of Vitamin D among subacute thyroiditis patients[27].

Orbach et al. found lower 25-hydroxy Vitamin D_3 levels in patients with AT than in healthy volunteers[28], and Kivity et al. found a significantly 25-hydroxy Vitamin D_3 deficiency in patients with HT compared to age-matched healthy individuals[29].

The sonographic characteristics of AT are well defined and used during US examinations. The histopathological findings and sonographic features in different stages of AT have been reported and used in clinical practice[22].

In the current study design, patients were staged according to the ultrasonographical morphological changes in the thyroid gland which occur even before anti-Tg Ab and TPO Ab levels were high enough in serum to diagnose AT.

According to Gierach and Junik , a lower level of Vitamin D was connected with a higher level of TSH, and they also found a strong, negative correlation between TSH and Vitamin D levels[30]. Moreover, there was a weak, negative correlation between antithyroid peroxidase antibody (anti-TPO) and anti-thyroglobulin antibody (anti-TG) and Vitamin D levels in females with HT regardless of Vitamin D status: < 20 ng/mL, 20-30 ng/mL, and > 30 ng/mL[30].

The main limitation of this study was the relatively small number of cases in the series.

In the study, the association between autoimmune thyroiditis and 25-hydroxy Vitamin D_3 levels was evaluated during different morphological changes in the thyroid gland by using ultrasonography. According to our results, 25-hydroxy Vitamin D_3 deficiency was detected even at early stage of thyroid gland destruction.

The association between significantly decreased 25 OH Vitamin D_3 concentrations and early morphological changes in the thyroid gland suggests that Vitamin D deficiency might play a role in the early development of thyroiditis.

STATEMENT OF ETHICS

This study was approved by the Medipol University Ethics Committee (10840098-604.01.01-E.28917).

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

MC was responsible for training LK, contributed to designing the research study, writing and editing the manuscript; LK was responsible for specimen handling, generating experimental data; TO was responsible for ultrasound examination of patients.

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