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The Relationship Between Thyroid Antibodies and Vitamin D Level in Primary Hypothyroidism

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ABSTRACT

Introduction: Vitamin D deficiency is a global health problem. Its deficiency has been reported to be associated with different autoimmune diseases. **Aim:** The aim of this study was to evaluate the relationship between vitamin D level and thyroid antibodies in autoimmune hypothyroidism. **Methods:** A total number of 150 individuals were enrolled in this study. They were divided into the fallowing groups: group I included 50 patients with autoimmune thyroid disease (AITD), group II included 50 patients without autoimmune thyroid disease. Group III included 50 apparently healthy participants representing a control group. All participants underwent a detailed clinical examination and laboratory tests including, 25 (OH) vitamin D, thyroid-stimulating hormone (TSH) and thyroid autoantibodies assessment, including anti-thyroid peroxidase antibodies (anti-TPO) and anti-thyroglobulin antibodies (anti-TG). **Results:** Serum levels of 25 (OH) vitamin D recorded a highly significant difference between the studies groups (20,76±6,31 ng/ml in group I vs. 24,37±9,05ng/ml in group II vs. 24,57±6,45ng/ml in group III, p<0,01). Moreover, there was a highly significant difference between patients with AITD and patients without AITD (20,76±6,31ng/ml vs. 24,37±9,05ng/ml, respectively; p<0,01). The concentration of anti-TPO and anti-TG antibodies were statistically significant higher in patients with vitamin D deficiency (p< 0,001). Serum TSH were significantly higher in group I (p< 0,001). **Conclusion:** Significantly low levels of vitamin D were documented in patients with AITD that were related to the presence of anti-thyroid antibodies and higher level thyroid-stimulation hormone (TSH), suggesting the involvement of vitamin D in the pathogenesis of AITD and the advisability of supplementation.

Keywords: autoimmune thyroid disease, vitamin D, thyroid autoantibodies, thyroid-stimulation hormone.

1. INTRODUCTION

Vitamin D deficiency is a global health problem. Prevalence of vitamin D deficiency or insufficiency is over a billion worldwide (1). The role of vitamin D has been evolving since the time of its discovery in the early 20th century from being a simple vitamin D to a steroid prohormone (2). Vitamin D deficiency has been shown to be associated with autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, multiple sclerosis, and type 1 diabetes, and that vitamin D supplementation prevents the onset and/or development of these autoimmune diseases (3). Autoimmune thyroid diseases (AITD), including Hashimoto's (HT) and Graves's (GD), are the most common organ-specific autoimmune disorders (4). These AITD are polygenic diseases resulting form a combination of genetic predisposition (thyroid-specific genes and immune-modulating genes) and environmental triggers (iodine, selenium, drugs, irradiation, smoking, infections, stress, etc), characterized by lymphocystic infiltration into the thyroid gland and production of thyroid-specific autoantibodies (4, 5). Both vitamin D and thyroid hormone bind to the steroid hormone receptors. Moreover, vitamin D mediates its effect by binding to vitamin D receptor (VDR), and activation of VDR-responsive genes. VDR gene polymorphism was found in association with autoimmune thyroid diseases (AITD) (6). Few studies were conducted to find any significant association between the levels of vitamin D and hypothyroidism and it's pathogenesis but yielded conflicting results. Kivity et al. in 2011 documented significantly low levels of 25(OH) vitamin D with autoimmune thyroid disease, whereas a study by Goswami et al. showed a weak association between 25(OH) vitamin D levels and thyroid peroxidase antibody (TPO-At) titers (7, 8).

2. AIM

The aim of this study was to evaluate the relation between vitamin D level, thyroid-stimulation hormone (TSH) and thyroid antibodies in primary hypothyroidism.

3. METHODS

The study is of a retrospective-prospective character, and it included a total of 150 individuals and conducted at the Radiology and Nuclear Medicine Clinic, Department for Thyroid Diseases, University Clinical Centre Tuzla, in period between January 2018 and December 2019. Participants were divided into the following groups: group I included 50 patients with autoimmune thyroid disease (AITD), group II included 50 patients without autoimmune thyroid disease (non-AITD). Group III included 50 apparently healthy participants representing a control group. All participants underwent a detailed clinical examination and laboratory tests including, 25 (OH) vitamin D, thyroid-stimulating hormone (TSH) and thyroid autoantibidies assessment, including anti-thyroid peroxidase antibodies (anti-TPO) and anti-thyroglobulin antibodies (anti-TG). The biochemical parameters were assayed in the Department of Thyroid Diseases attached to our clinic. Thyroid-stimulating hormone (TSH) were measured with a fluoroimmunometric assay (DELFIA) on the machine Wallac delfia fluorometer. TSH levels between 0,63-4,19mIU/L were regarded normal.

Anti-TPO and anti-TG were tested by radioimmunoassay (RIA). The measuring of the serum anti-TPO and anti-TG concentration was performed on Wallac Wizard 1470 automatic gamma counter. Positive anti-TPO, and anti-TG were defined as a value greater than > 60 IU/ml. Elevated serum levels of thyroid autoantibodies were used for diagnosis of AITD. Electro-chemiluminescence binding assay (ECLIA) was used for vitamin D (total 25 hydroxy vitamin D) on the mashine Cobas e 411 Rosche. Vitamin D deficiency is defined as a 25 (OH) vitamin D below 20 ng/ml and vitamin D insufficiency as 25(OH) vitamin D of 21-29 ng/ml. Levels of 25(OH) vitamin D > 30 ng/ml are considered to be optimal.

The statistical analysis was conducted with SPSS version 23.0 for Windows. The descriptive analysis was applied for all date processing. In the statistic data processing, the fallowing were applied: Tukey's post hoc test, Spearman rank correlation coefficient. The level of check importance was set on 5% ($p < 0,05$).

4. RESULTS

This study enrolled 150 participants who were subdivided into three groups: group I included 50 patients recently diagnosed with AITD (evidence by elevated anti-TPO, and anti-TG serum levels), group II included 50 patients without autoimmune thyroid disease (non-AITD). Group III included 50 apparently healthy particpans representing a control group with normal clinical examination and were not complaining of either any chronic medical diseases, or history of thyroid diseases or any chronic illness that may interfere with results to be obtained. Also, these participants were not on any sort of vitamin D supplementations.

VitaminD sufficiency (n (%))	Patients group					
	With autoimmune hypothyroidism		Without autoimmune hypothyroidism		Control group	
	f	%	f	%	f	%
Deficitent	34	68%	19	38%	12	24%
Insufficient	6	12%	14	28%	31	62%
Sufficient	10	20%	17	34%	7	14%

Table 1. Comparation between patients with AITD, patients without AITD, and control group regarding Vitamin D sufficiency

Variable	Patients group	μ ± σ	F-test	df	P
Age	AITD	49.32 ± 14.57 [a,b]	3.875	(2;147)	0.023
	Non-AITD	54.20 ± 13.45[a]			
	Control group	45.34 ± 19.22[b]			
TSH	AITD	7.57 ± 8.68[a]	15.302	(2;147)	0.000
	Non- AITD	2.82 ± 1.60[b]			
	Control group	2.53 ± 0.87[b]			
Anti-TPO	AITD	1715.58 ± 969.79[a]	149.380	(2;147)	0.000
	Non-AITD	25.31 ± 10.51[b]			
	Control group	45.37 ± 66.85[b]			
Anti-TG	AITD	293.47 ± 429.50[a]	20.434	(2;147)	0.000
	Non-AITD	16.56 ± 7.4[b]			
	Control group	20.92 ± 14.15[b]			
25(OH) vitamin D	AITD	20.76 ± 6.31[a]	4.212	(2;147)	0.017
	Non-AITD	24.37 ± 9.05[b]			
	Control group	24.57 ± 6.45[b]			

Table 2. The assessment of significant difference in the analyzed parameters between patients with AITD with non-AITD, and each of them with control group(post-hoc Tukey test), AITD-autoimmune thyroid disease, Non-AITD-without autoimmune thyroid disease

Total amount of 150 participants were included in our research, aging 11 to 83, and 89,33% were women.

Serum levels of 25 (OH) vitamin D recorded a significant difference between the studied groups (20.76±6.31 ng/ml in group I vs. 24.37±9.05 ng/ml in group II vs. 24.57±6.45ng/ml in group III, $p<0.05$).

Regarding vitamin D sufficiency, it was revealed that 25 (OH) vitamin D was deficient in 68%, insufficient in 12% and sufficient in 20% of the group I vs. deficient in 38%, insufficient in 28% and sufficient 34% of the group II. On the contrary, 25 (OH) vitamin D was deficient in only 24%, insufficient in 62%, and sufficient in 14% of control group, as illustrated in (Table 1).

The post-hoc Tukey test was used to determine the existence of significant differences in the analyzed parameters between patients with AITD with non-AITD, and each of them with control group. Age distribution showed statistical difference between with non-AITD patients and control group. Moreover, there was a highly

TSH			Anti-TPO	Anti-TG	25(OH) D
TSH	Correlation Coefficient	1.000	0.476	0.392	-0.212
	p-value		0.000	0.000	0.009
	N	150	150	150	150
Anti-TPO	Correlation Coefficient		1.000	0.761	-0.225
	Sig. (2-tailed)			0.000	0.006
	N		150	150	150
Anti-TG	Correlation Coefficient			1.000	-0.328
	Sig. (2-tailed)				0.000
	N			150	150
Vitamin D	Correlation Coefficient				1.000
	Sig. (2-tailed)				
	N				150

Table 3. The assessment of correlation between the TSH, Anti-TPO, Anti-TG and vitamin D serum levels (Spearman coefficient), TSH-thyroid-stimulating hormone, Anti-TPO-anti-thyroid peroxidase antibodies, Anti-TG-anti-thyroglobulin antibodies

significant difference between patients with AITD and patients with non- AITD regarding TSH (7.57±8.68 mU/l in patients with AITD vs. 2.82±1.60 mU/l in patients non AITD, p<0.05) and between patients with AITD and control group (7.57±8.68 mU/l vs. 2.53±0.87 mU/l), but no significant statistical difference between patient with non-AITD and control group regarding TSH (2.82±1.60 mU/l vs. 2.53±0.87 mU/l).

Regarding anti-TPO level and anti-TG level, there was a significant difference between patients with AITD and patients with non-AITD (1715.58± 969.79 IU/ml; 293.47±429.50 IU/ml vs. 25.31±10.51 IU/ml; 16.56±7.4IU/ml and between patients with AITD and control group (1715.58±969.79 IU/ml; 293.47± 429.50IU/ml vs. 45.37±66.85 IU/ml; 20.92±14.15 IU/ml), but no significant difference between patients with non-AITD and control group (25.31±10.51 IU/ml; 16.56± 7.4 IU/ml vs. 45.37± 66.85 IU/ml; 20.92±14.15 IU/ml)

Regarding 25 (OH) vitamin D level, there was a significant difference between patients with AITD and patients with non AITD (20.76±6.31 vs. 24.37±9.05 ng/ml, respectively, p<0,05), as well as between patients with AITD and control group (20.76±6.31 vs. 24.57±6.45 ng/ml, respectively, p<0.05), but no significant difference between patients with non-AITD and control group (24.37±9.05 ng/ml vs. 24.57±6.45 ng/ml), as illustrated in (Table 2).

Moreover, vitamin D deficiency was more frequent in patients with AITD (68%) versus in patients without AITD (38%).

The Spearman test was used to investigate correlation of nonparametric variables including vitamin D, TSH and autoantibodies. The results revealed there was a significant negative correlation between the vitamin D with TSH level (p=0.009, r=-0.212) and between vitamin D with anti-TG level (p=0.000, r=-0.328) and anti-TPO level (p=0.006, r=-0.225), as show in (Table 3).

5. DISCUSSION

Thyroid diseases are among the most common endocrine abnormalities, and AITDs are perhaps the most prevalent autoimmune diseases (9, 10). AITD are relatively common organ-specific autoimmune disorders that cause diseases ranging in severity from hypothyroidism to hyperthyroidism (11). As an immune modulator, vitamin D is involved in the onset and development of AITD (7, 12).

Majority of the participants in our study were females (90%). This finding was similar to that of Mackawy et al. (13). He stated that serum vitamin D levels were significantly more decreased in females than males. This was in accordance with our finding. Although several authors have reported that vitamin D levels did not differ significantly between males and females (13, 14, 15).

In our study, there was a highly significant difference regarding 25 (OH) vitamin D level among the studied groups; 25(OH) vitamin D level was 20.76±6.31 ng/ml in patients with AITD versus 24.37±9.05 ng/ml in patients with non-AITD versus 24.57±6.45 ng/ml in control group. Regarding vitamin D status, it was deficient in 68% in patients with AITD, 38% in patients with non-AITD, whereas it was deficient in 24% in control group.

This is in agreement with Friedman study (16) who explained the low levels of vitamin D in patients with hypothyroidism by two mechanisms. First, it may be owing to poor absorption of vitamin D from the intestine. Second, the body may not activate vitamin D properly. Importantly, both vitamin D and thyroid hormone bind to similar receptors called steroid hormone receptors. A different gene in the vitamin D receptor was shown to predispose people to AITD.

Also, Kivity et al. (17) reported that the prevalence of vitamin D deficiency was significantly higher in 50 patients with AITD compared with 98 healthy individuals (72% vs. 30,6%, respectively, p<0.001). Vitamin D deficiency was also found to be correlated with the presence of anti-thyroid antibodies (p=0.01), suggesting the involvement of vitamin D in the pathogenesis of AITD.

Moreover, we found that 68% of patients with AITD had vitamin D deficiency, whereas only 38% of patients without AITD had vitamin D deficiency. In our study, we found that serum TSH was significantly higher in patients with AITD than patients without AITD. However , a study Shin et al (18) did not find any significant difference. This could be due to a different sample population.

Our study showed a significant negative correlation between vitamin D and TSH level (p=0.009).

An experimental study by Byron Richards (18) studied, that was showed a lack of vitamin D leading to the possibility of increased thyroid -stimulating hormone,

so the significant (p<0,05) negative correlation between vitamin D and TSH indicates the correlation between hypothyroidism and vitamin D.

However, we are documented a statistically negative correlation between vitamin D levels and thyroid antibodies, this result comes in agreement with Hosny, et al. (18), who found a negative relation between serum 25 (OH) vitamin D and anti-TPO and anti-TG. As well as, Khare, et al. (19), noted that the amount of serum 25(OH)D did not differ significantly between positive TPO-Ab and negative TPO-Ab subjects, which is in accordance with our results. Also, Yasmeh, et al. (20), have found no association with anti-TPO positivity and a poor inverse correlation between the levels of Vitamin D and anti-TPO has been found, which is contradictory to our findings. In other studies, several researchers found that the incidence of 25(OH)D, deficiency among TPO-Ab positive was significantly higher that in TPO-Ab negative hypothyreoid patients (7, 21). The contradictory and different results of the study are partly due to inter-assay and inter-laboratory variability in 25 (OH) D levels of Vitamin D, seasonal differences in 25(OH) blood samples.

6. CONCLUSION

Significantly lower levels of vitamin D were documented in patients with AITD. Deficiency of vitamin D was linked to the presence of thyroid antibodies and abnormal thyroid functions. Further studies are required to determine whether vitamin D deficiency is the causal factor or the consequence of primary hypothyroidism, and to provide insight into the efficacy and safely of vitamin D as a therapeutic tool for AITD.

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