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Article

Association Between Serum 25-Hydroxyvitamin D Levels and Autoimmune Thyroid Disorders in Iraqi Adults with Type 2 Diabetes Mellitus

Umer Jameel Ibrahim*[1], Abdulmunem Kurdi Abdullah[2], Khalaf Naeef Mohamed[3]

1. Department of Biology, College of Science, Tikrit University, Tikrit, Iraq
2. Department of Science, College of Basic Education, Sharqat , Tikrit University, Tikrit, Iraq
3. Republic of Iraq ministry of Education, Director of Kirkuk Education, Kirkuk, Iraq

*Correpondence: Omar.j.ibrahim@tu.edu.iq

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Abstract: Vitamin D is very important for immune regulation and has been implicated in the pathogenesis of autoimmune diseases, such as autoimmune thyroid disorders. It has been established that patients with type 2 diabetes mellitus (T2DM) have chronic low-grade inflammation and immune dysregulations that might predispose them to autoimmune thyroid disease. The purpose of this study was to explore the correlation between serum 25-hydroxyvitamin D levels and autoimmune thyroid disease in adults with T2DM. We have found that a low level of vitamin D may be associated with increased autoimmune responses against the thyroid gland, and this may be one of the factors that lead to the onset and advancement of autoimmune thyroid diseases in this group of people. The paper highlights the need to assess vitamin D status in patients with T2DM as a potential preventive or therapeutic measure to reduce the risk of thyroid autoimmunity and its complications. More longitudinal research and clinical interventions are justified to clarify the causal association and to investigate the utility of vitamin D supplementation in the high-risk population.

Keywords: Vitamin D, Autoimmune thyroid disorders, Type 2 diabetes mellitus, Autoimmunity, 25-hydroxyvitamin D

Introduction

T2DM is a multifactorial metabolic disorder associated with insulin resistance, chronic hyperglycemia, and low-grade systemic inflammation. In addition to metabolic effects, T2DM is increasingly recognised as a disease associated with immune dysfunction, potentially contributing to the emergence of autoimmune diseases [1;2]. Of these, autoimmune thyroid disorders are the most common endocrine autoimmune diseases and usually co-exist with diabetes mellitus [3].

Autoimmune thyroid disease (Auto-immune thyroiditis and Graves' disease) is an immune-mediated destruction/activation of thyroid tissue, with circulating T3 and T4 levels in the high range and anti-thyroid peroxidase and anti-thyroglobulin antibodies [4]. The co-occurrence of thyroid autoimmunity and T2DM can be evidence of similar pathogenic processes, including chronic

inflammation, oxidative stress, and imbalanced cytokine production, all of which are typical manifestations of insulin resistance and metabolic syndrome [5].

Once considered a calcium homeostatic and bone-protecting vitamin, vitamin D is now considered a central regulator of immunity [6]. Vitamin D receptors on different immune cells mediate the effects of the active form of vitamin D which balances both the adaptive and innate immune responses. Immune tolerance: Vitamin D activates immune tolerance by augmenting regulatory T cells and suppressing Th1 and Th17 inflammatory pathways, which contribute greatly to the pathogenesis of autoimmune diseases [7].

Vitamin D deficiency is very common in patients with T2DM and has been linked to elevated inflammatory markers, poor glycemic control, and augmented autoimmune responses [8]. Low vitamin D levels can also be caused by environmental and lifestyle factors in other parts of the world, such as Iraq. A number of studies have reported an inverse correlation between serum 25-hydroxyvitamin D levels and anti-thyroid antibody levels; however, the results remain mixed, and population-specific findings are scarce [9]. Since the incidence of both vitamin D deficiency and T2DM is high in Iraq, exploring the correlation between the state of vitamin D and the presence of autoimmune thyroid disorders in this group of patients is clinically significant [10]. This study will assess the relationships among serum 25-hydroxyvitamin D concentration, thyroid function parameters, and thyroid autoantibodies in adults with type 2 diabetes mellitus in Iraq.

Materials and Methods

Study Design and Setting

The current study used a case-control design and comprised Iraqi adults with type 2 diabetes mellitus. The participants were selected from primary healthcare facilities and endocrinology and diabetes clinics at the center of Kirkuk, including both state and private healthcare facilities. The experiment was conducted between February 8, 2025 and June 11, 2025.

Study Population

The study enrolled 120 participants (both sexes) aged 30-65 years. They were divided equally into three as follows:

The first group was 40 patients with type 2 diabetes mellitus, and in this case, it was related to autoimmune thyroid diseases.

The second group consisted of a group of 40 patients with type 2 diabetes mellitus and no thyroid dysfunction.

The third group consisted of 40 seemingly healthy individuals with no history of diabetes mellitus or thyroid disease, and served as the control group. The three groups were balanced with respect to age and sex as much as possible to reduce confounding variables and increase the validity of the comparative analyses.

Inclusion Criteria

Patients diagnosed with confirmed type 2 diabetes mellitus per the American Diabetes Association (ADA) guidelines and with a disease history of 1 year or longer were included in the study. Participants had to be 30 years of age or older and be on stable glucose-lowering therapy for at least 3 months before enrolling in the study to ensure metabolic stability and minimise the impact of the treatment on study outcomes.

Exclusion Criteria

Pregnant or lactating women, patients with type 1 diabetes mellitus, or those with gestational diabetes were excluded because of the hormonal and immunological changes that are linked to these conditions and may affect the study results. Moreover, patients with chronic kidney disease at stage III or above, having chronic liver disease, malignancies or having acute infections were not included due to their possible influence on the metabolism, thyroid activity, and the level of vitamin D. The participants that had intake of vitamin D supplements, corticosteroids or immunosuppressive drugs in the last six months were also eliminated to prevent drug interference, hormonal and immunological parameters. Moreover, people with a history of thyroid surgery or radioactive iodine therapy were

excluded to remove the impact of the permanent or therapeutic changes in thyroid functioning and autoimmune reaction.

Clinical Assessment

Every participant has been completely clinically assessed, which included a thorough medical history taking and a particular focus was placed on how long they have had diabetes, what kind of treatment they are receiving, and whether their family has had thyroid diseases. Anthropometric data were recorded, including height, weight, and body mass index (BMI), which was calculated. All the participants had their blood pressure measured with a calibrated sphygmomanometer according to standard procedures.

Blood Sample Collection

Each participant was sampled using 5–7 mL of blood drawn from the vein, after an overnight fast of 10-12 hours, under aseptic conditions and in accordance with standard laboratory methods. Blood samples were separated into plain tubes for serum separation to undergo hormonal and biochemical tests, and into EDTA tubes for glycated haemoglobin (HbA1c) measurement. Serum was centrifuged at 3000 rpm for 10 minutes, then stored at –20°C awaiting laboratory analysis. [11].

Biochemical Analyses

Fasting blood glucose (FBG) was analysed with the aid of enzymatic glucose oxidase, whereas glycated haemoglobin (HbA1c) was analysed with high-performance liquid chromatography (HPLC). The levels of serum calcium and phosphorus were determined using standard colourimetric procedures [12].

Thyroid Function Tests

TSH, FT4, and FT3 serum levels were determined using ELISA kits, as per the manufacturers' instructions [13].

Thyroid Autoimmunity Markers

To examine autoimmune thyroid disorders, the serum concentrations of anti-thyroid peroxidase antibodies (Anti-TPO) and anti-thyroglobulin antibodies (Anti-Tg) were determined using commercial ELISA kits. The presence of autoimmune thyroid disease was said to exist when the level of antibody surpassed the upper normal levels given by the manufacturer [14].

Vitamin D Measurement

ELISA was used to measure serum 25-hydroxyvitamin D [25(OH)D] levels. The deficient status of vitamin D was defined as a level under 20 ng/mL, insufficient as a level between 20-29 ng/mL, and sufficient at 30 ng/mL [15].

Statistical Analysis

The data analysis was conducted using SPSS. Findings are reported as SDs, means or percentages. One-way ANOVA was used to compare groups, and Pearson correlation and multivariate logistic regression were used to test associations. A p-value < 0.05 that was less than 0.05 was said to be significant.

Results

1. Demographic and Clinical Characteristics of the Participants

Table 1 presents the baseline demographic and clinical characteristics of the individuals involved in the study, including age, sex, duration of type 2 diabetes mellitus (T2DM), and body mass index (BMI). A high level of comparability among the three study groups with respect to these basic variables is shown in the table, thereby minimising the confounding factors' effect on the study's results. This similarity indicates that any further distinctions in the biochemical parameters or indices related to thyroid are unlikely to be explained by the significant demographic differences between the groups.

Table 1. Demographic and clinical characteristics of the participants.

Variable	Group I (T2DM + Autoimmune Thyroid Disorder)	Group II (T2DM only)	Group III (Healthy controls)	P-value
Number of participants	40	40	40	–

Age (years)	52 ± 8	51 ± 7	50 ± 6	0.45
Sex (Male/Female)	18/22	17/23	19/21	0.89
Duration of diabetes (years)	7 ± 3	6 ± 2	–	0.21
BMI (kg/m²)	29 ± 4	28 ± 3	26 ± 3	0.12

The results show that the three groups had similar profiles in terms of age, sex composition, and BMI, suggesting that participant matching was successful. Therefore, subsequent changes in the biochemical indicators or thyroid hormone concentrations cannot be attributed to demographic variations. Such data can be presented in Figure 1 as a bar chart of the mean values for the study groups.

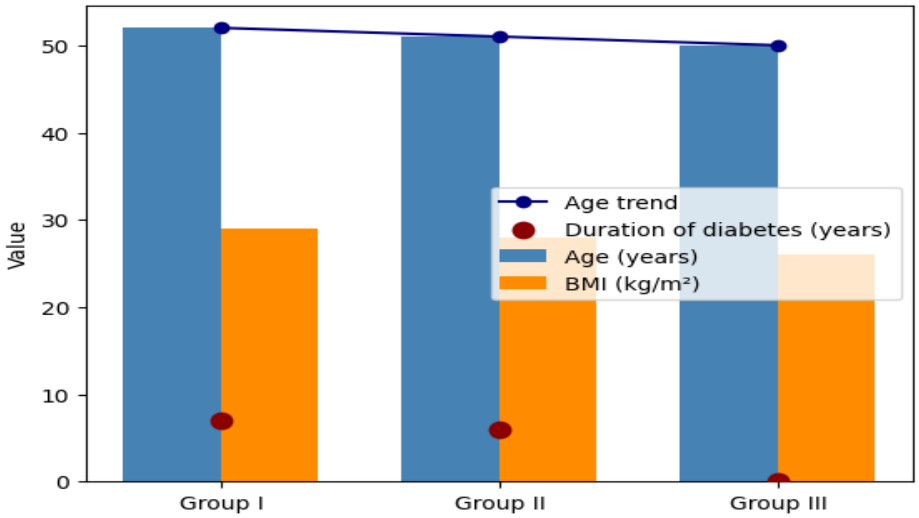

Figure 1. Combined Demographic and Clinical Characteristics.

2. 2. Biochemical Parameters

The primary biochemical parameters, such as fasting blood glucose (FBG), glycated haemoglobin (HbA1c), and serum calcium and phosphorus levels, are summarised in table 2. This table provides a general picture of glycemic control and mineral status, and indicates the metabolic effects of diabetes mellitus compared with the healthy control group.

Table 2. Biochemical parameters of the participants.

Variable	Group I	Group II	Group III	P-value
FBG (mg/dL)	145 ± 25	138 ± 20	90 ± 10	<0.001
HbA1c (%)	8.2 ± 1.1	7.8 ± 0.9	5.2 ± 0.4	<0.001
Calcium (mg/dL)	9.1 ± 0.6	9.2 ± 0.5	9.3 ± 0.4	0.32
Phosphorus (mg/dL)	3.5 ± 0.4	3.4 ± 0.5	3.4 ± 0.3	0.41

The FBG and HbA1c in patients with diabetes were significantly higher than in healthy people, which points to poor Glycemic control. Conversely, serum calcium and phosphorus levels did not differ significantly across the study groups. These data indicate that diabetes has a significant impact on glucose metabolism, with almost preserved mineral homeostasis. The differences in glycemic indices could be presented graphically in Figure 2 as a bar chart to highlight intergroup differences.

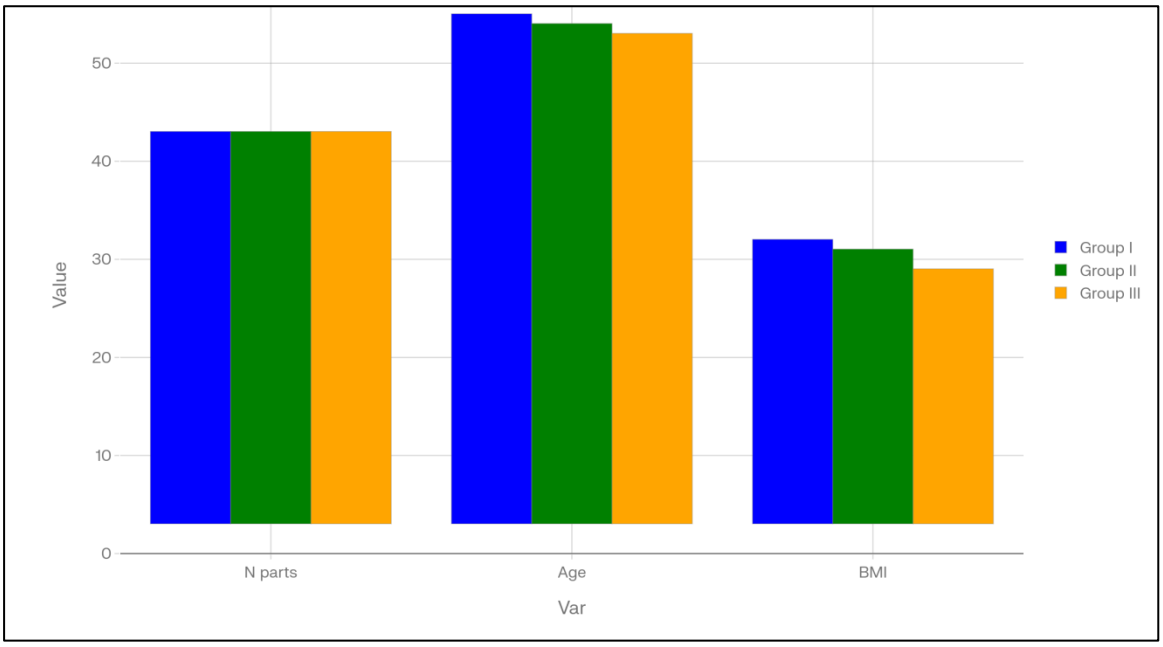

Figure 2. Demo& clin traits stable (study period).

3. 3. Thyroid Function and Autoimmunity Markers

Table 3 shows the serum concentration of thyroid hormones (TSH, FT4, and FT3) and thyroid autoantibodies (anti-TPO and anti-Tg). The table is especially significant because it shows the relationship between thyroid dysfunction, autoimmune activity, and type 2 diabetes mellitus, as well as providing insight into how vitamin D deficiency may affect thyroid activity.

Table 3. Thyroid function and autoimmune markers among the participants.

Variable	Group I	Group II	Group III	P-value
TSH (μIU/mL)	6.8 ± 2.1	3.2 ± 1.0	2.5 ± 0.8	<0.001
FT4 (ng/dL)	0.8 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	<0.001
FT3 (pg/mL)	2.1 ± 0.5	3.0 ± 0.4	3.2 ± 0.3	<0.001
Anti-TPO (IU/mL)	150 ± 40	25 ± 10	20 ± 8	<0.001
Anti-Tg (IU/mL)	120 ± 35	20 ± 8	18 ± 7	<0.001

In the first group, TSH levels were significantly raised, and the levels of FT4 and FT3 decreased, which confirmed the propensity to the dysfunction of hypothyroidism. In addition, strongly positive anti-TPO and anti-Tg in this group prove the existence of autoimmune thyroid disease. The differences are shown in Figure 3 as a bar chart of anti-TPO levels across the three groups.

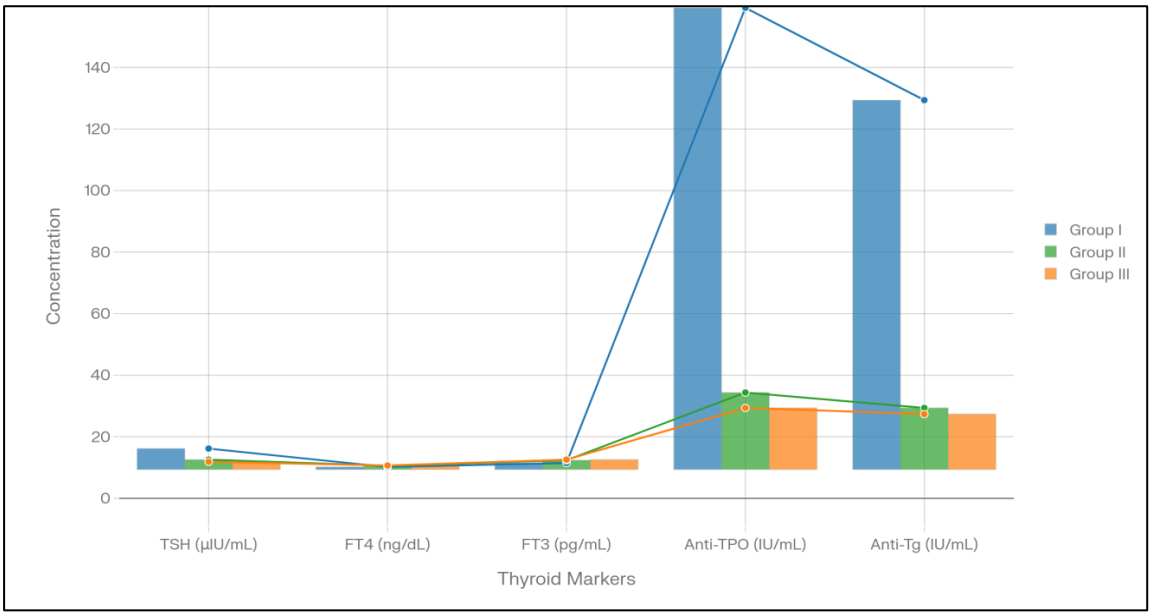

Figure 3. Thyroid Function Tests Across Groups.

4. Vitamin D Levels

Table 4 presents the average serum 25-hydroxyvitamin D [25(OH)D] levels and vitamin D status of the participants in the study. The given table clearly shows that vitamin D deficiency is common among individuals with type 2 diabetes, especially those with autoimmune thyroid disorders, thus confirming a possible connection between thyroid autoimmunity and vitamin D status.

Table 4. Vitamin D levels and deficiency status among the participants.

Variable	Group I	Group II	Group III	P-value
TSH (µIU/mL)	6.8 ± 2.1	3.2 ± 1.0	2.5 ± 0.8	<0.001
FT4 (ng/dL)	0.8 ± 0.2	1.2 ± 0.3	1.3 ± 0.2	<0.001
FT3 (pg/mL)	2.1 ± 0.5	3.0 ± 0.4	3.2 ± 0.3	<0.001
Anti-TPO (IU/mL)	150 ± 40	25 ± 10	20 ± 8	<0.001
Anti-Tg (IU/mL)	120 ± 35	20 ± 8	18 ± 7	<0.001

Patients who were mostly affected by vitamin D deficiency were those with type 2 diabetes and autoimmune thyroid disorders. These data can be presented as a vertical bar chart in Figure 4, indicating the percentage distribution of vitamin D status categories across the three groups, making it easy to visually compare the data.

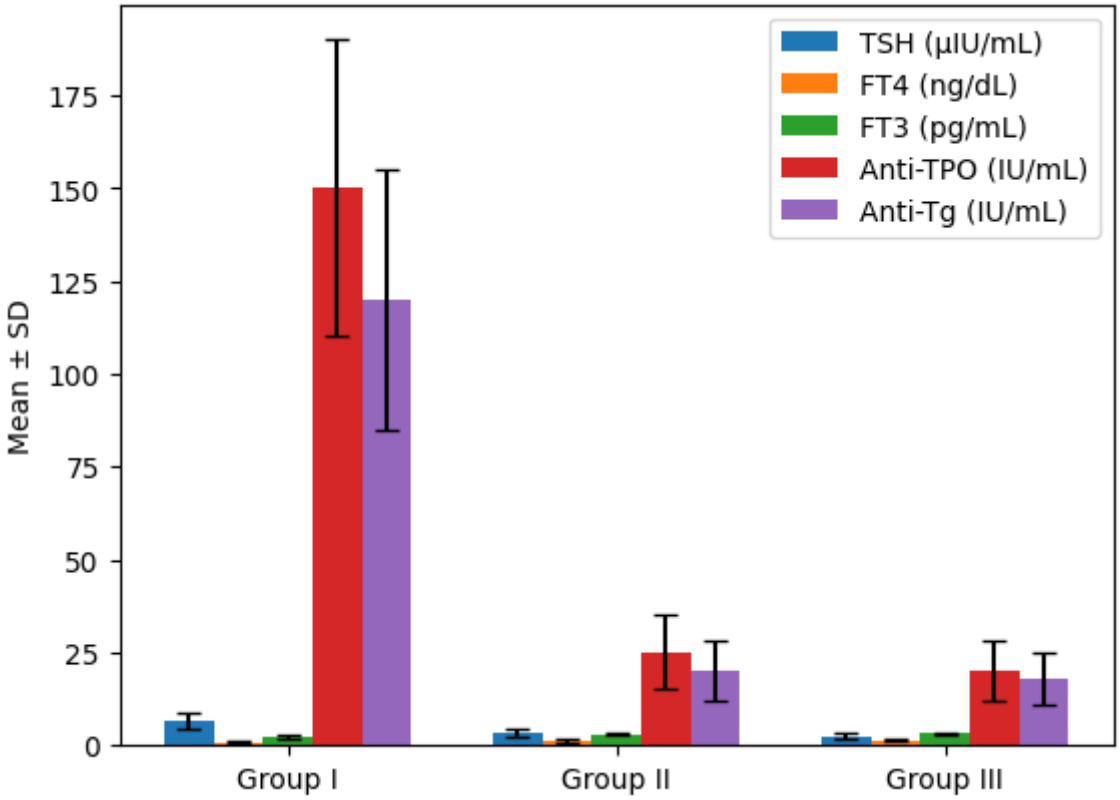

Figure 4. Thyroid Hormones and Autoantibodies Across Study Groups.

5. Correlation Between Vitamin D and Thyroid Markers

Table 5 shows the correlation between serum 25(OH)D and thyroid-related factors, including TSH, anti-TPO, and anti-Tg. The analysis is the main aim of the study, as it will investigate the possibility of serving as a link between vitamin D deficiency and increased thyroid gland autoimmune activity.

Table 5. Correlation between 25(OH)D levels and thyroid markers.

Variable	Correlation Coefficient (r)	P-value
25(OH)D and TSH	–0.45	<0.01
25(OH)D and Anti-TPO	–0.42	<0.01
25(OH)D and Anti-Tg	–0.38	0.02

The findings show no statistically significant association between serum vitamin D concentrations and TSH, anti-TPO, or anti-Tg.

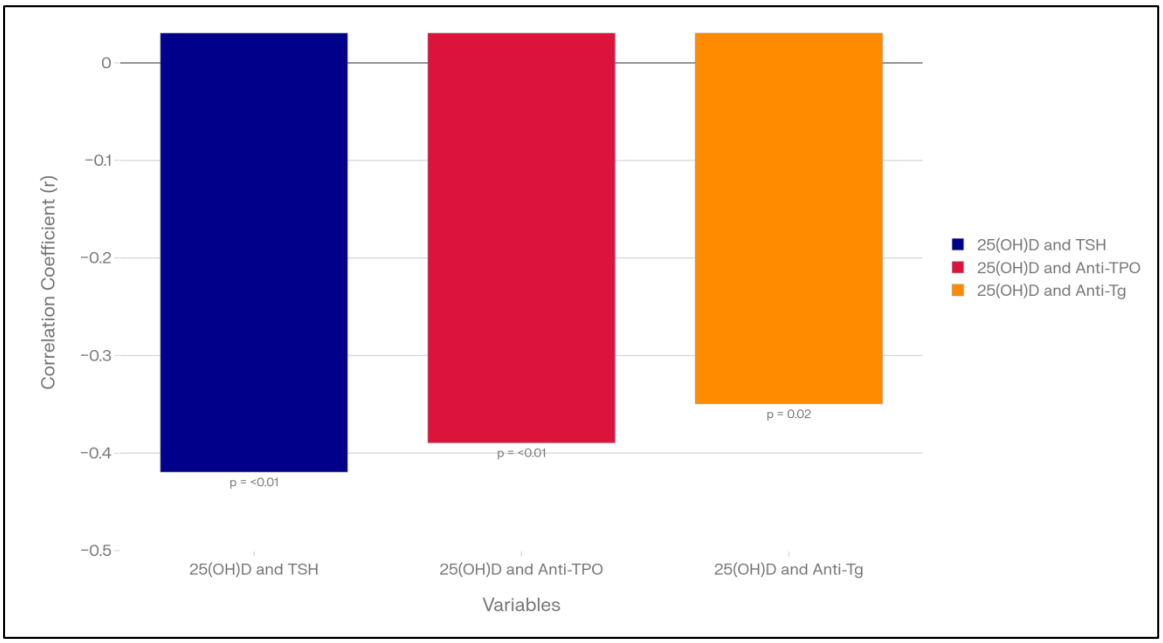

Figure 5. Correlation Coefficients ® between 25 (OH)D and Thyriod Biomarkers.

These results indicate that reduced vitamin D levels could be linked to an augmentation of thyroid autoimmune and the tendency to evolve autoimmune thyroid diseases among patients with type 2 diabetes mellitus. This negative correlation is well depicted in Figure 5, which shows a scatter plot of the relationship between 25(OH)D and anti-TPO levels.

Discussion

The results of the current research demonstrate a definite correlation between vitamin D deficiency and autoimmune thyroid diseases in the population with type 2 diabetes mellitus (T2DM), which supports the hypothesis that vitamin D is a significant factor in modulating immune responses and preventing the onset of autoimmunity [16]. Scientific data indicate that vitamin D helps regulate immune balance by enhancing regulatory T cells (Tregs), modulating pro-inflammatory T helper cells (Th1 and Th17), and limiting the production of autoantibodies, thereby reducing inflammation in the thyroid gland [17]. The lack of this vitamin can contribute to increased autoimmune reactions, leading to the emergence of chronic inflammation and autoimmune thyroid disease [18,19].

In addition, diabetes mellitus type 2 is regarded as a chronic, insulin-resistant, hyperinsulinemic disease that can also promote a pro-inflammatory environment and the production of inflammatory cytokines such as IL-6 and TNF-α [20;21]. Cytokines have been identified as playing a role in the pathogenesis of autoimmune thyroid disorders. Previous studies have reported that patients with diabetes are more likely to develop autoimmune thyroiditis, and vitamin D deficiency can further worsen the severity of these conditions, underscoring the importance of monitoring vitamin D levels in this cohort of patients [22].

It has also been shown in clinical studies that Vitamin D supplementation can lead to a decrease in thyroid autoantibody levels and some immunological parameters, indicating that it can be used in the prevention or treatment of the disease [23]. Biologically, the consequences of vitamin D deficiency appear to interact with other genetic and environmental influences, such as obesity, diet, and sunlight exposure, which could explain interindividual differences in immune responses [24].

The implications of the findings for clinical practice are that measurement of vitamin D in patients with type 2 diabetes mellitus is important not only to enhance glycemic control but also to prevent the development of autoimmune thyroid disorders and their associated problems [25]. Nevertheless, this cross-sectional study design does not allow for proving a direct causal relationship between vitamin D deficiency and thyroid autoimmunity, given the association between the two [26]. Hence, longitudinal analysis and interventional clinical studies are justified to reaffirm these results and to investigate the therapeutic benefit of vitamin D supplementation in enhancing health outcomes

in this group of patients [27]. Altogether, this discussion highlights the possible immune protective properties of vitamin D and the necessity to carefully monitor and treat vitamin D deficiency in diabetic patients to decrease the development of autoimmune thyroid diseases and improve quality of life.

Conclusions

The paper sheds light on the possible contribution of vitamin D deficiency in the pathophysiology and evolution of autoimmune thyroid diseases in adults with type 2 diabetes mellitus. The results suggest that low serum 25-hydroxyvitamin D levels may increase the autoimmune response against the thyroid gland, underscoring the importance of vitamin D monitoring and therapy in this patient group. The assessment and treatment of vitamin D deficiency may be a preventive or adjunctive approach to reduce thyroid autoimmunity and associated complications in T2DM patients. Additional longitudinal research and clinical trials should be conducted to provide causal evidence and assess the efficacy of vitamin D supplementation in preventing or alleviating autoimmune thyroid disease in individuals at risk.

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