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Research paper

Autoimmune thyroiditis in children and adolescents with type 1 diabetes mellitus is associated with elevated IgG4 but not with low vitamin D

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ABSTRACT

OBJECTIVE: To assess levels of vitamin D and of immunoglobulin G subclasses in children and adolescents with type 1 Diabetes Mellitus with or without autoimmune thyroiditis. DESIGN: Among 213 patients with type 1 diabetes, the cases with thyroid-specific autoantibodies formed Group 1 [n=19, M/F: 7/12, median age 13 years (10.1-14.7)]. Nineteen age-, gender-, and diabetes duration-matched cases with type 1 diabetes without any other systemic disease were designated as controls [Group 2, M/F: 7/12, median age 12.9 years (10.5-14.9)]. RESULTS: Levels of thyroid hormones, vitamin D, total IgG and IgG subclasses, as well as IgG subclasses/total IgG ratios were similar between the groups. Five cases (26%) in Group 1 had IgG4 levels > + 2 SDS, whereas there were no such cases in Group 2 (p=0.046). These five patients had similar clinical features but higher median IgG4 levels and IgG4/Total IgG ratios compared to the subjects with IgG4 levels < + 2 SDS in Group 1 and Group 2. CONCLUSIONS: There was no difference of vitamin D levels between the groups. Only a small percentage of patients with type 1 diabetes also having autoimmune thyroiditis had elevated serum IgG4 levels, revealing the heterogeneity of autoimmune thyroiditis and existence of IgG4 thyroiditis in the pediatric age group. Total IgG, the other IgG subclasses, and vitamin D levels did not differ in patients with autoimmune thyroiditis and type 1 diabetes compared to those suffering only from type 1 diabetes.

Key words: Autoimmune thyroiditis, Immunoglobulin G subclasses, Type 1 diabetes, Vitamin D

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INTRODUCTION

Autoimmune thyroiditis is the most frequent autoimmune disease accompanying type 1 diabetes in up to 30% of children and adolescents suffering from type 1 Diabetes Mellitus.[1-3] Typical features of the

disease are presence of thyroid specific antibodies (antithyroid peroxidase antibodies and antithyroglobulin antibodies) in serum and varying degrees of thyroid dysfunction.[1] While the exact pathogenesis is unclear, female gender, certain HLA types, polymorphisms in the *CTLA-4* gene, chromosomal diseases, puberty, smoking, endocrine disruptors, and Yersinia infections are among the risk factors for autoimmune thyroiditis.[4] Increasing age, longer duration of diabetes, and presence of anti-GAD antibody have also been found to be associated with autoimmune thyroiditis in patients with type 1 diabetes.[5,6]

Vitamin D is a fat-soluble vitamin which is mainly involved in calcium/phosphate homeostasis and bone mineralization. However, increasing evidence points to the role of vitamin D in cell proliferation and differentiation, regulation of the immune system, glucose metabolism, and cardiovascular health.[7] Recently, children and adults with Hashimoto's thyroiditis were found to have significantly lower 25(OH) vitamin D [25(OH)D] levels and higher frequency of vitamin D deficiency compared to controls.[8-10] On the other hand, the role of vitamin D levels in Hashimoto's thyroiditis accompanying type 1 diabetes, for which deficiency of vitamin D and genetic variations in vitamin D-related genes were proposed as being predisposing factors, has not yet been investigated.[11]

Recently, in adults who required total thyroidectomy, a subtype of Hashimoto's thyroiditis was described, termed "IgG4 thyroiditis", which is characterized by an abundance of IgG4-positive plasma cells and fibrosis in the thyroid tissue, lower female/male ratio, higher percentage of diffuse low echogenicity at ultrasonography, more rapid clinical progression, and higher serum levels of IgG4 and thyroid autoantibodies.[12] There are a limited number of studies regarding IgG subclasses in children with type 1 diabetes or autoimmune thyroiditis. To the best of our knowledge, their association with autoimmune thyroiditis and type 1 diabetes has not been evaluated in relevant studies.

In the present study, we aimed to put forward the hypothesis that low vitamin D levels and/or elevated IgG4 levels might be associated with autoimmune thyroiditis in patients with type 1 diabetes.

METHODS

Subjects and Setting

Among 213 type 1 diabetes patients regularly followed in the two pediatric endocrinology clinics in the city of Gaziantep, Turkey, 25 cases (11.7%) were found to have autoimmune thyroiditis as determined by presence of thyroid-specific autoantibodies including antithyroid peroxidase antibodies (TPOAb, reference range 0-35 IU/mL) and antithyroglobulin antibodies (TGAb, reference range 0-40 IU/mL).[13] Six of them could not finally be included despite several unsuccessful attempts to make contact, and the remaining 19 cases formed Group 1 (type 1 diabetes + autoimmune thyroiditis). Among the subjects with type 1 diabetes who were enrolled in the study, age-, gender-, and diabetes duration-matched cases without any other systemic disease were designated as controls (n=19, Group 2). Furthermore, the participants of Group 1 were assigned to two subgroups: those who had an IgG4 level $>+2$ SD score (Subgroup 1) and those with an IgG4 level $<+2$ SD score (Subgroup 2). Informed consent was obtained from both the parents and subjects. The study protocol was in compliance with the Declaration of Helsinki and approved by the institutional ethical review board.

Clinical and Laboratory Procedures

During a period free of infection or inflammation, all the subjects underwent physical examination (including assessment of weight, height, pubertal state, and thyroid size) and blood sampling following descriptive data collection (including exposure to smoke, family history of autoimmune thyroiditis, insulin dose, and L-thyroxine use). None of the patients was using vitamin D preparations or smoking. Regular exposure of participants to second-hand smoke within the previous six months was considered as passive smoking.[14] Thyroid size was evaluated clinically according to the World Health Organization criteria.[15] Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Auxological data were converted to standard deviation scores (SDS) by using data from the National Health and Nutrition Examination Survey.[16]

Complete blood count and hemoglobin A1c analyses were performed on the day of evaluation. Serum

specimens for free thyroxine (fT4), thyroid-stimulating hormone (TSH), TgAb, TPOAb, calcium, phosphorus, alkaline phosphatase, parathormone, 25(OH) vitamin D, total immunoglobulin G (IgG), and IgG subclasses were stored at -20°C. Biochemical and hormonal analyses were performed using Abbott Architect ci8200 (Abbott Laboratories, Illinois, USA) by using standard methodology. Serum IgG, IgG1, IgG2, IgG3, and IgG4 were measured by the nephelometric method (Beckman Coulter Immage 800, Beckman Coulter International SA, Nyon, Switzerland) using commercially available kits (Binding Site Group Ltd, Birmingham, UK). The interpretation of levels of IgG and IgG subclasses was carried out using reference values generated from healthy Turkish children.[17] Ultrasonography was performed to establish the size and echogenicity characteristics of the thyroid in patients with autoimmune thyroiditis. Thyroid sizes of the subjects were converted to SDS by using data reported by Kurtoglu et al.[18]

Statistical Analyses

The data were analyzed using computer software SPSS 15.0 (Chicago, Illinois, USA). All continuous variables were compared with nonparametric tests (Mann-Whitney U-test and Kruskal-Wallis test), since the Shapiro-Wilk test demonstrated that few numerical variables were normally distributed among the groups compared. Comparison of study variables was first made between Groups 1 and 2. The chi-square or Fisher's exact test were used to compare categorical variables. Univariate correlation analysis was performed using Spearman's rank correlation coefficient. A p-value of <0.05 was chosen to represent statistical significance. Comparisons between subgroups and controls were made using the Kruskal-Wallis test followed by the Mann-Whitney U-test with Bonferroni correction. A *p*-value of <0.0167 was considered to represent a statistically significant difference: *p*-value $= 0.05 \times 2/k(k-1)$, where k represents the number of comparisons.[19] All data were presented as median (25^{th} – 75^{th} percentiles) or n (%).

RESULTS

Group 1 consisted of 19 subjects with type 1 diabetes and autoimmune thyroiditis [(M/F: 7/12, median age 13 years (10.1-14.7), median disease duration 2.9 years (0.4-5.3)]. Group 2 included 19 control cases with type 1 diabetes [M/F: 7/12, median age 12.9 years (10.5-14.9), median disease duration 3.2 years (1.5-5.1)]. The two groups were found to have similar clinical characteristics including known risk factors for autoimmune thyroiditis (Table 1). None of the patients was obese or an active smoker. All of the subjects were receiving basal-bolus insulin regimen. One of the patients was using L-thyroxine (2.7 μg/kg/d) due to overt hypothyroidism.

Table 1. Clinical characteristics of the subjects with (Group 1) and without (Group 2) autoimmune thyroiditis

	Group 1 (n=19)	**Group 2 (n=19)**	*p*
Age (years)	13.0 (10.1 – 14.7)	12.9 (10.5 – 14.9)	0.931
Gender (female)	12 (63%)	12 (63%)	0.999
Age at diagnosis (years)	8.1 (5.8 – 12.8)	9.0 (7.8 – 11.6)	0.665
Diabetes duration (years)	2.9 (0.4 – 5.3)	3.2 (1.5 – 5.1)	0.729
Weight SDS	-0.04 (-0.84 – 0.56)	-0.07 (-0.71 – 0.67)	0.840
Height SDS	-0.42 (-0.89 – 0.46)	-0.88 (-2.10 – -0.33)	0.146
BMI SDS	0.29 (-0.15 – 0.70)	0.25 (-0.58 – 1.32)	0.885
Exposure to smoke	9 (47%)	5 (26%)	0.138
Family history of autoimmune thyroiditis	5 (26%)	2 (11%)	0.209
Goiter	2 (10%)	0 (0%)	0.486
Pubertal subjects	13 (68%)	13 (68%)	0.999
Daily insulin dose (units/kg/d)	1 (0.7 – 1)	1.1 (0.8 – 1.6)	0.189

Results are given as median (25th - 75th percentiles) or n (%). SDS: standard deviation score; BMI: body mass index.

One subject from Group 1 had iron deficiency anemia. None of the subjects had leukocytosis, neutrophilia, lymphocytosis or thrombocytosis. Group 1 and Group 2 were similar regarding hemoglobin A1c [10.3% (8 – 11.3) vs. 8.7% (7.7 – 10.1), p=0.181], fT4 [1.05 ng/dL (1.01 – 1.11) vs. 1.06 ng/dL (0.99 – 1.13), p=0.931], and TSH levels [1.9 mIU/L (1.1 – 2.5) vs. 1.4 mIU/L (1.1 – 2.7), p=0.418]. As expected, levels of thyroid-specific autoantibodies were significantly higher in Group 1 [TgAb, 28.5 IU/mL (2.95 – 154.9) vs. 0.86 IU/mL (0.77 – 1.61), p=0.001; TPOAb, 173.7 IU/mL (47.4 – 399.9) vs. 0.18 IU/mL (0.06 – 0.25), p=0.001]. Positivity rates of TPOAb, TgAb, and TPOAb&TgAb in Group 1 were 94.7% (n=18), 52.6% (n=10), and 47.3% (n=9), respectively. Calcium, phosphorus, and alkaline phosphatase levels were all within normal ranges and did not differ between the groups (data not shown). In Group 1, ultrasonographic evaluation of the thyroid (n=14, 73.7%) yielded a volume SDS of 0.01 (-0.92 – 0.42), coarse echogenicity in nine cases (64.3%), and normal echogenicity in five subjects (35.7%).

Serum vitamin D levels of the subjects were all below 20 ng/mL in both groups, indicating that none of the patients was vitamin D sufficient (Table 2).[20] Levels of vitamin D, total IgG and IgG subclasses, ratios of IgG subclasses to total IgG, and number of subjects with IgG1, IgG2, and IgG3 levels > +2 SD were similar between the groups. IgG4 levels of the patients are shown in Figure 1. Five patients (26%) in Group 1 had IgG4 levels above the upper limit of

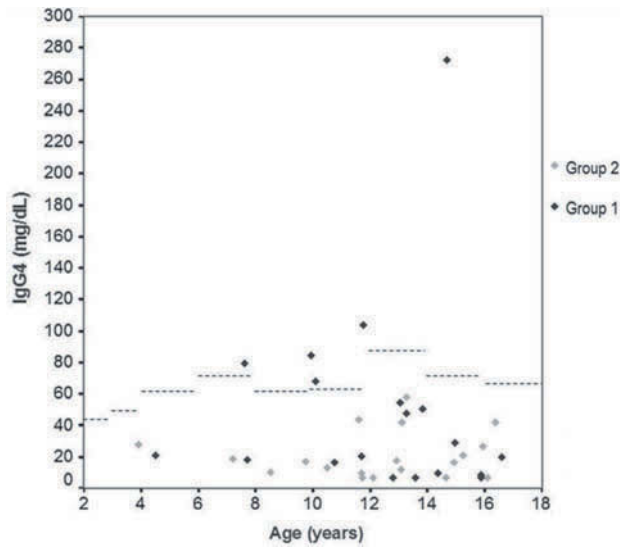

Figure 1. Levels of IgG4 in cases with (Group 1) and without (Group 2) autoimmune thyroiditis. Dotted lines indicate +2 SD of relevant age groups.

Table 2. Vitamin D, parathormone, and immunoglobulin results of the groups

	Group 1 (n=19)	**Group 2 (n=19)**	*p*
25-OH Vitamin D (ng/mL)	10.7 (8.8 – 13.3)	9.8 (7.7 – 11)	0.284
PTH (pg/mL)	48.1 (35.7 – 54.0)	47.2 (29.9 – 65.8)	0.988
Total IgG (mg/dL)	1100 (948 – 1300)	1020 (790 – 1240)	0.123
IgG1 (mg/dL)	727 (640 – 843)	680 (508 – 806)	0.191
IgG1/Total IgG	0.68 (0.62 – 0.73)	0.67 (0.62 – 0.69)	0.773
IgG1 level > + 2 SDS	3 (16%)	1 (5%)	0.604
IgG2 (mg/dL)	266 (211 – 399)	222 (184 – 299)	0.096
IgG2/Total IgG	0.25 (0.22 – 0.32)	0.25 (0.21 – 0.28)	0.370
IgG2 level > + 2 SDS	7 (37%)	2 (10%)	0.124
IgG3 (mg/dL)	82 (57 – 129)	64 (53 – 107)	0.506
IgG3/Total IgG	0.075 (0.046 – 0.107)	0.077 (0.059 – 0.105)	0.563
IgG3 level > + 2 SDS	9 (47%)	5 (26%)	0.179
IgG4 (mg/dL)	20.8 (9.6 – 68.2)	17.2 (9.8 – 27.6)	0.130
IgG4/Total IgG	0.025 (0.009 – 0.055)	0.017 (0.009 – 0.033)	0.223
IgG4 level > + 2 SDS	5 (26%)	0 (0%)	0.046

Results are given as median (25th - 75th percentiles) or n (%). PTH: parathormone; IgG: immunoglobulin G; SDS: standard deviation score.

age-related reference range, while all of the subjects in Group 2 had normal IgG4 levels (p=0.046). Correlation analyses in Group 1 revealed that vitamin D and IgG4 levels were not correlated with any of the clinical and laboratory variables studied.

Clinical and radiological features and vitamin D levels were similar among the subgroups and controls. Levels of thyroid-specific autoantibodies did not differ between the subgroups but were higher compared to those of controls. Positivity rates of antibodies among Subgroup 1 and Subgroup 2 were similar: TPOAb, 80% (n=4) vs. 100% (n=14), p=0.263; TgAb, 60% (n=3) vs. 50% (n=7), p=0.999; TPOAb and TgAb, 40% (n=2) vs. 50% (n=7), p=0.999, respectively. IgG4 levels and IgG4/Total IgG ratios in Subgroup 1 were higher than those of Subgroup 2 and controls (Table 3).

DISCUSSION

To the best of our knowledge, only varying degrees of deficiencies have been reported in studies regarding serum IgG subclasses in patients with type 1 diabetes.[21] In the present study, we found that serum IgG4 levels were above the upper limit in nearly one fourth of patients with type 1 diabetes and autoimmune thyroiditis, while none of the serum IgG4 levels exceeded the upper limits of normal in age-, sex-, and diabetes duration-matched control cases without autoimmune thyroiditis. This finding provides further evidence that autoimmune thyroiditis is a heterogene-

Table 3. Comparisons between patients with type 1 diabetes, autoimmune thyroiditis, and an IgG4 level > +2 SD score (Subgroup 1), patients with type 1 diabetes, autoimmune thyroiditis, and an IgG4 level < +2 SD score (Subgroup 2) and patients with type 1 diabetes without autoimmune thyroiditis (Group 2).

	Group 1 (n=19)			***p values***			
	Subgroup 1 (n=5)	**Subgroup 2 (n=14)**	**Group 2 (n=19)**	**All three groups**	**Subgroup 1 vs. Subgroup 2**	**Subgroup 1 vs. Group 2**	**Subgroup 2 vs. Group 2**
Age (years)	10.1 (8.8-13.2)	13.4 (11.5-15.2)	12.9 (10.5-14.9)	0.317	0.138	0.271	0.500
Gender (female)	3 (60%)	9 (64%)	12 (63%)	0.999	0.999	0.999	0.947
Age at diagnosis (years)	7.3 (6.1-8.8)	9.2 (5.6-13)	9.0 (7.8-11.6)	0.419	0.579	0.126	0.999
Diabetes duration (years)	3.6 (1.9-4.8)	2.1 (0.4-7.4)	3.2 (1.5-5.1)	0.747	0.547	0.749	0.536
Exposure to smoke	4 (80%)	5 (36%)	5 (26%)	0.108	0.293	0.092	0.734
Family history of autoimmune thyroiditis	3 (60%)	2 (14%)	2 (10%)	0.066	0.161	0.071	0.999
Goiter	0 (0%)	2 (14%)	0 (0%)	0.243	0.964	N/A	0.336
Coarse echogenicity[α]	3 (75%)	6 (60%)	-	0.999	N/A	N/A	N/A
Thyroid volume SDS[α]	0.1 (0.01-1.26)	-0.63 (-1.24-0.42)	-	0.188	N/A	N/A	N/A
Pubertal subjects	3 (60%)	10 (71%)	13 (68%)	0.999	0.999	0.999	0.999
25(OH) Vitamin D (ng/mL)	11.3 (8.7-16.2)	10.5 (8.7-13.3)	9.8 (7.7-11)	0.473	0.578	0.271	0.423
TgAb (IU/mL)	12.1 (1.9-80.0)	34.8 (5.4-298.2)	0.86 (0.77-1.61)	0.001	0.459	0.001	0.001
TPOAb (IU/mL)	46.3 (23.9-593.0)	192.7 (67.7-365.6)	0.18 (0.06-0.25)	0.001	0.517	0.001	0.001
IgG4 (mg/dL)	84.5 (73.9-188)	18.9 (8.1-33.7)	17.2 (9.8-27.6)	0.002	0.001	0.001	0.855
IgG4/Total IgG	0.080 (0.060-0.114)	0.017 (0.007-0.036)	0.017 (0.009-0.033)	0.002	0.001	0.001	0.855

Results are given as n (%) and median (25th - 75th percentile); [α]: Ultrasonography could not be performed in 1 case from Subgroup 1 and 4 cases from Subgroup 2. SDS: standard deviation score; TgAb: antithyroglobulin antibodies; TPOAb: antithyroid peroxidase antibodies; IgG: immunoglobulin G; N/A: not applicable.

ous disease and suggests that IgG4 thyroiditis might exist in children with type 1 diabetes as well.

IgG4 thyroiditis was first described by Li *et al.* following the observation of a high number of IgG4-positive plasma cells in thyroid tissue in some of the adults who required total thyroidectomy due to Hashimoto's thyroiditis (mean disease duration 9.6 years). Serum IgG4 levels, which could be measured in a subset of patients, TgAb and TPOAb levels, L-thyroxine doses, number of patients with subclinical hypothyroidism, and male/female ratio were higher and disease duration was shorter in the latter cases compared to those of subjects with non-IgG4 thyroiditis.[12] Histopathological evaluation was not made in the present study given the lack of clinical necessity and ethical issues. Our subgroup analysis revealed that subjects with type 1 diabetes and autoimmune thyroiditis accompanied by elevated IgG4 levels had similar clinical, imaging, and laboratory features with respect to the other subgroup despite a tendency towards higher rates of family history of autoimmune thyroiditis and exposure to smoke and lower levels of thyroid autoantibodies.

The role of smoking in thyroid autoimmunity is complex. In adults, a relationship between active smoking and Hashimoto's thyroiditis has been suggested. However, active smoking, but not passive smoking, has recently been reported to be protective against thyroid-specific autoantibody positivity.[22-24] As far as we are aware, no such data exist regarding children or adolescents with type 1 diabetes. In our cases, none of whom were smokers, passive smoking was more common in Group 1. Interestingly, this difference seems to be associated with subjects with elevated IgG4. In contrast with recent reports, despite being statistically insignificant, thyroid-specific autoantibody median levels were lower in subjects with elevated IgG4 (Subgroup 1), of whom 80% were exposed to smoke, compared to that of subjects with normal IgG4 (Subgroup 2). Smoking status of IgG4 thyroiditis patients, in whom mean thyroid-specific autoantibody levels were notably high, was not mentioned in the original report.[12] IgG4 levels were analyzed in several studies performed in nicotine consumers or active smokers and no high levels were detected.[25-27] Further studies in children with autoimmune thyroiditis using biomarkers of passive smoking including cotinine, nicotine, or exhaled carbon monoxide would be of benefit.

IgG4 is the least abundant subclass in blood and has the weakest capability of interacting with classical Fc gamma receptors and C1q.[17,28] Accordingly, it is generally non-pathogenic. However, purified IgG4 from humans with an endemic form of pemphigus foliaceus has resulted in a similar clinical picture in mice.[28] In IgG4-related sclerosing disease, which is a steroid-responsive systemic autoimmune syndrome characterized by tumor-like lymphoplasmacytic infiltration of various tissues, sclerosis and elevated serum IgG4 level, and possibly an IgG4 thyroiditis subgroup of Hashimoto's thyroiditis, elevated blood levels of IgG4 appear to be the consequence of increased numbers of IgG4-positive plasma cells in the tissues rather than the cause of disease.[12,28] One third to half of patients with type 1 diabetes patients and thyroid autoantibody positivity have been reported to develop subclinical or overt hypothyroidism during follow-up.[29,30] As was also shown in adults with Hashimoto's thyroiditis, elevated IgG4 levels in our study group may be associated with the frequency and tempo of progression to hypothyroidism in type 1 diabetes.[12]

Among the endocrine glands, the pancreas and pituitary are also involved in IgG4-related sclerosing disease. Autoimmune pancreatitis is one of the commonest presentations, characterized by mild abdominal symptoms, obstructive jaundice, and enlarged pancreas.[31] Headache and visual disturbances with or without hypopituitarism are associated with IgG4-related autoimmune hypophisitis. Cranial imaging reveals sellar mass and/or thickened pituitary stalk.[32,33] Our cases with elevated IgG4 levels were not evaluated thoroughly regarding these conditions; however, absence of relevant symptoms or laboratory findings (e.g. central hypothyroidism) militates against pancreatic or pituitary involvement.

In the present study, all of the vitamin D levels were found to be below the lower limit of sufficiency (20 ng/mL). A recent evaluation of healthy Turkish children between 1 and 16 years of age in Ankara revealed that 14.5% of cases had 25(OH)D levels below 20 ng/mL.[34] However, a much higher percentage of children and adolescents with type 1 diabetes

(71%) from the same city were found to have 25(OH) D levels below this limit.[35] Whatever the underlying cause, vitamin D deficiency did not appear to be associated with autoimmune thyroiditis in subjects with type 1 diabetes in our study, unlike other studies. Camurdan et al reported that vitamin D deficiency was nearly four times more prevalent and 25(OH)D levels were significantly lower (31.2±11.5 nmol/L) in 78 children with Hashimoto's thyroiditis (hypothyroidism, 6.4%; subclinical hypothyroidism, 15.4%, euthyroidism, 78.2%) compared to controls (n=74, 57.9±19.7 nmol/L) (conversion factor for 25(OH) D: ng/mL=nmol/L/2.496).[8] In agreement with our findings, a longitudinal follow-up of adult subjects revealed that prevalence of vitamin D deficiency comparable between cases who developed TPOAb during follow-up compared to those who did not, resulting in the suggestion that vitamin D deficiency is not associated with early thyroid autoimmunity.[36]

In conclusion, the present study demonstrated that a subset of cases with type 1 diabetes and autoimmune thyroiditis have elevated IgG4 levels, suggesting existence of IgG4 thyroiditis. Total IgG, other IgG subclasses, and vitamin D levels were found to be not associated with autoimmune thyroiditis in type 1 diabetes. In further studies, the prognostic value of increased serum IgG4 levels for thyroid dysfunction in type 1 diabetes should be investigated.

CONFLICT OF INTEREST

None.

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