

Clinical Significance of IgG4 Serum Concentration in Graves' Disease

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Abstract

Elevated immunoglobulin G4 (IgG4) serum antibodies are an important feature of IgG4-related disease. However, IgG4 antibodies can play a role in autoimmune thyroid disorders. In this study, we aimed to evaluate the impact of serum IgG4 levels on clinical features of Graves' disease (GD). We recruited 60 patients with GD (48 patients without thyroid eye disease, 12 patients with moderate-to-severe Graves' orbitopathy [GO], and 25 healthy control subjects). The prevalence of high IgG4 serum concentration was 4.2% among GD patients without GO and 33.33% in patients with moderate-to-severe GO. The group with GO had significantly higher median IgG4 levels (87.9 mg/dL) than the control group (41.2 mg/dL, $P = 0.034$) and the GD without GO group (30.75 mg/dL, $P < 0.001$). Patients with thyroid nodules had lower IgG4 levels than patients without thyroid nodules, but the difference was not statistically significant (35.7 [24.8; 41.53] mg/dL vs. 43 [30.1; 92.7] mg/dL, $P = 0.064$). IgG4 as a diagnostic tool for moderate-to-severe GO had the following parameters: area under the curve (AUC): 0.851 ($P < 0.001$), at the cut-off value of 49 mg/dL, negative predictive value: 100%, positive predictive value: 48%, sensitivity: 100%, specificity: 73%. There were no significant differences between the high and normal IgG4 groups in thyroid hormones, antithyroid antibodies, and ultrasound features. Serum IgG4 levels are associated with some of the clinical features of GD and can help in the diagnostic process of the disease. More research is needed to better understand the pathophysiology of IgG4 involvement in GD.

Keywords

IgG · IgG4 · Graves' disease · Thyroid eye disease · IgG4-related disease

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1. Introduction

Immunoglobulin G4 (IgG4)-related disease (IgG4-RD) is a relatively new, systemic, fibro-inflammatory disorder, which, for the first time, was described in 2003 (Kamisawa et al. 2003). It is characterized by high IgG4 serum concentration, swelling, pathological mass or nodules of the affected organs, which on histopathological examination demonstrate infiltration by IgG4⁺ plasmacyte cells, storiform fibrosis, and obliterative phlebitis (Umehara et al. 2021). In the case of the thyroid gland, Riedel thyroiditis is considered a manifestation of IgG4-RD, as it fulfills all the comprehensive clinical, radiological, and histopathological diagnostic criteria (Katz and Stone 2022). However, there is also evidence that IgG4 antibodies play a role in other inflammatory thyroid diseases. This group of disorders is called IgG4-related thyroid disease (IgG4-RTD). Pathologies from the IgG4-RTD spectrum typically lack systemic involvement and do not fulfill all the strict histopathological criteria of IgG4-RD. Besides Riedel thyroiditis, authors distinguish three more subtypes of IgG4-TRD. Those disorders are the fibrosing variant of Hashimoto thyroiditis (HT), IgG4-related HT, and Graves' disease (GD) with elevated/high IgG4 concentration (Kottahachchi and Topliss 2016; Rotondi

et al. 2019). From the aforementioned group of diseases, the smallest amount of evidence is available for GD with elevated IgG4 levels. This variant of GD occurs in 10.3% of patients with GD. It is especially prevalent in patients with Graves' orbitopathy (GO), which is the most common cause of orbital inflammation in adults (Nowak et al. 2024). In total, 65% of the GD patients with elevated IgG4 serum concentration have GO. Patients with elevated IgG4 GD also present different characteristics compared to patients with normal IgG4 GD (Olejarski et al. 2021). Most of the data about the role of IgG4 in GD comes from Asia. So far, only three studies have been performed in Europe or America. In this study, we aimed to assess: (1) the prevalence of GD with elevated/high IgG4 serum concentration; (2) the differences between this GD variant and the classic variant with normal IgG4 serum concentration; and (3) the associations between IgG4 level and thyroid hormones, antithyroid antibodies, and ultrasonographic features.

2. Methods

The study was conducted in a single tertiary reference endocrine center. It was a cross-sectional study. All adult patients with GD who did not undergo definitive treatment for Graves' hyperthyroidism (radioiodine, thyroidectomy) were recruited into the study. We applied the following

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exclusion criteria: (1) history of treatment with radioiodine or thyroidectomy, (2) systemic immunosuppressive or immunomodulatory treatment at the time of admission or in the previous 6 months, (3) chronic kidney disease stages 4 and 5, (4) liver failure, (5) active neoplastic disease or any suspicion of malignancy, (6) active acute or chronic infections, (7) any immunodeficiency disorder, and (8) pregnancy. The age- and sex-matched control group comprised individuals selected to closely resemble the demographic characteristics (age and sex) of the participants in the study group. These individuals were healthy volunteers with no diagnosed thyroid disorders, as confirmed by laboratory tests and thyroid ultrasound examinations.

We recruited 60 patients with GD and 25 healthy control subjects. The GD without orbitopathy subgroup consisted of 48 patients without overt thyroid eye disease recruited from our outpatient clinic. Patients with minor ocular symptoms (mild eyelid retraction, without edema, with a clinical activity score [CAS] of 0) were also included in the group. In this group, 29 patients were hyperthyroid and 19 were euthyroid after methimazole treatment. The second group consisted of 12 patients with moderate-to-severe GO as defined by EUGOGO (Bartalena et al. 2021), of whom 3 were hyperthyroid and 9 were euthyroid after methimazole treatment. Those patients were mainly recruited from our in-patient center. A detailed composition of the studied group can be seen on the flowchart presented in Figure 1.

We have taken fasting venous blood samples from each patient. The laboratory assessment included: (1) hormonal function of the thyroid gland—free triiodothyronine (fT3), free thyroxine (fT4), and thyroid-stimulating hormone (TSH), (2) thyroid antibodies—anti-TSH receptor antibodies (TRAb), anti-thyroid peroxidase antibodies (TPOAb), anti-thyroglobulin antibodies (TgAb), (3) basic markers of inflammation—C-reactive protein (CRP) and complete blood count (CBC), and (4) basic markers of liver and kidney function (aspartate transaminase [AST], alanine transaminase [ALT]), creatinine.

We measured fT3, fT4, TSH, TPOAb, TgAb, CRP, ALT, AST, and creatinine on the COBAS 8000 analyzer (Roche Diagnostics, Switzerland). TRAb levels were assessed by the radioimmunological method with a commercially available radioimmunoassay kit (Brahms GmbH, Germany). We did the CBC analysis with the use of the flow cytometry-based hematology analyzer Sysmex-XN 1000 (Sysmex Europe GmbH). We measured total IgG by a turbidimetric assay (Roche Diagnostics, Germany). To measure IgG4 levels, we used an immune-enzymatic assay using commercially available ELISA kits (Sunredbio, China). We defined high IgG4 as >135 mg/dL. This cut-off level is unanimously recognized in IgG4-RD and has also been used in previous research on the role of IgG4 in GD and other autoimmune thyroid diseases.

We performed a thyroid ultrasound on every patient. We calculated the thyroid volume of each thyroid lobe with the use of the following equation: $0.52 \times \text{length} \times \text{width} \times \text{depth}$.

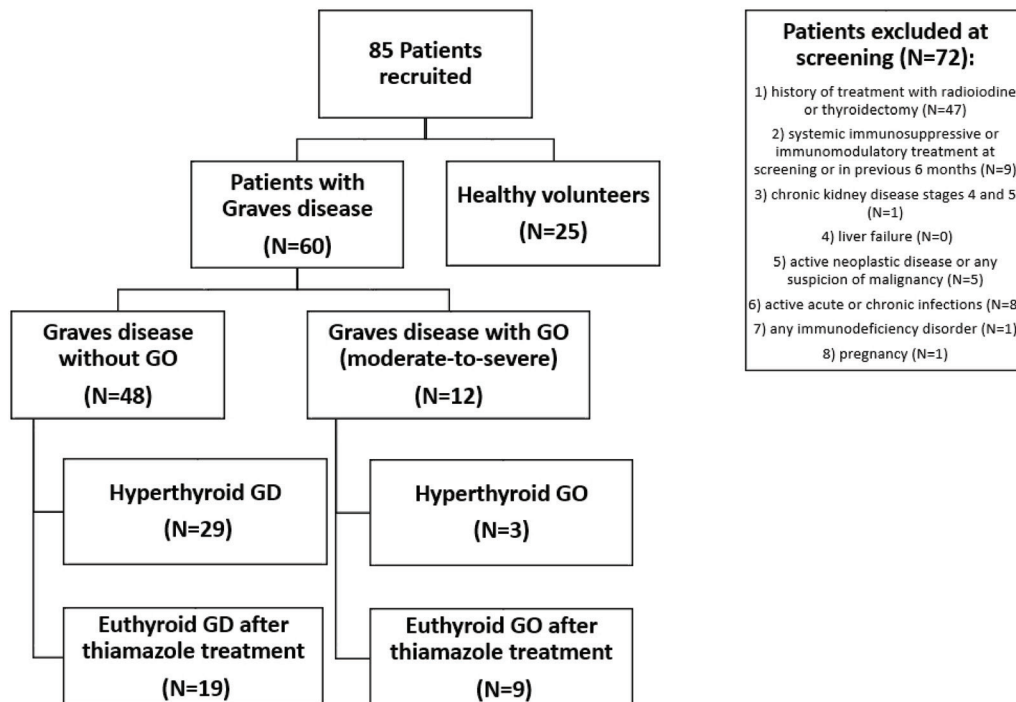


Fig 1. Flowchart displaying the composition of the studied group. GD, Graves' disease; GO, Graves' orbitopathy.

Total thyroid volume was calculated by adding the volumes of both lobes. We assessed the thyroid echogenicity and scored it in the following way: 0—normal echogenicity, 1—slight hypoechogenicity, and 2—deep hypoechogenicity. We evaluated intrathyroidal blood flow and scored it the following way: 0—normal intrathyroidal blood flow, 1—mildly increased intrathyroidal blood flow, and 2—severely increased intrathyroidal blood flow. We noted the presence of any thyroid nodules >5 mm and used the biggest of the three diameters (length/width/depth) for further calculations. The ultrasound examinations were performed with the use of Aixplorer by Supersonic Imagine (Aix-en-Provence, France).

Every patient signed a written informed consent to participate in the study. The research was conducted in accordance with the Declaration of Helsinki (Sawicka-Gutaj et al. 2022). The local Bioethics Committee at the Poznan University of Medical Sciences reviewed and approved the study (decision numbers: 774/20 and 209/23).

The statistical analyses were performed using PQ Stat v. 1.8.6 (PQStat Software, Poland). We used the Shapiro–Wilk to test for normality in each subgroup. Subsequently, we assessed the equality of variances with the Levene's test. Groups of variables with normal distribution and equal variances were compared using the Student's *t*-test. We used the Mann–Whitney *U* test for comparisons of two groups of ordinal variables or continuous variables without a normal distribution or unequal variances. The Kruskal–Wallis ANOVA was used for comparisons between multiple groups of continuous variables without a normal distribution or ordinal variables. If the *P*-value of the Kruskal–Wallis test was <0.05, we used the Dunn–Bonferroni test in the *post hoc* analysis. To compare qualitative data, we used the Fischer's exact test. In the case of comparison of qualitative data between multiple groups, we used the Fischer's exact test and applied the Bonferroni correction to the test. We presented data as mean ± standard error (SE) or median (first quartile; third quartile). The *P*-value was considered significant at the level of <0.05. All tests were two-tailed. We calculated the Pearson's tests to check for correlations between continuous variables with a normal distribution and the Spearman's rank correlation to check for correlations between ordinal variables and continuous variables without a normal distribution. "*R*" was used as the abbreviation for the Pearson Correlation Coefficient and "*ρ*" was used as abbreviation for the Spearman's Rank Correlation Coefficient.

3. Results

In our group of patients with GD, the prevalence of high IgG4, defined as ≥135 mg/dL, was 10% (6/60 subjects). It was notably higher in the group of patients with moderate-to-severe GO (33.33%; 4/12) and lower among patients without GO (4.2%; 2/48, one patient from the euthyroid and one from the

hyperthyroid subgroup). There were no statistically significant differences between the high IgG4 group and the normal IgG4 groups in terms of thyroid hormones, antithyroid antibodies levels, and ultrasound features. The groups also did not differ in age, gender distribution, CBC parameters, and routine kidney and liver test results (Table 1). The GD and control groups did not differ in total IgG (11.25 [10.32; 12.58] g/L vs. 11.14 [9.89; 13.21] g/L, *P* = 0.8) and IgG4 serum concentrations (35.9 [26.18; 88.58] mg/dL vs. 41.2 [37.3; 67.2] mg/dL, *P* = 0.43). We divided the GD group according to orbitopathy status. The group with moderate-to-severe GO had significantly higher IgG4 levels ([87.9 mg/dL] than the control group [41.2 mg/dL, *P* = 0.034], and the GD without GO group (30.75 mg/dL, *P* < 0.001) (Figure 2). There was no statistically significant difference between the GO, GD, and control groups in total IgG levels.

We performed a subgroup analysis and divided patients according to their hormonal status (euthyroid or hyperthyroid). Patients with hyperthyroid and euthyroid GD did not differ in IgG4 levels (33 [23.78; 58.3] mg/dL vs. 52.4 [29.45; 95.6] mg/dL, *P* = 0.061), despite huge differences in antithyroid antibodies and thyroid hormone levels (Table 2).

Total IgG correlated negatively with age in the control group (*R* = (–)0.426, *P* = 0.043) but not in the GD group. A correlation between IgG4 and age was not observed. Among GD patients, IgG correlated positively with fT3 (*ρ* = 0.353, *P* = 0.006), fT4 (*ρ* = 0.387, *P* = 0.002), while IgG4 correlated negatively with fT4 (*ρ* = (–)0.260, *P* = 0.045) and TRAb (*ρ* = (–)0.270, *P* = 0.037) (Table 3). However, on subgroup analysis after dividing the group into GO and GD without GO, the only correlation that remained statistically significant was the positive correlation of IgG with fT3 (*ρ* = 0.296, *P* = 0.041), and fT4 (*ρ* = 0.303, *P* = 0.036), in the GD without GO subgroup and a negative correlation between IgG and TRAb (*ρ* = (–)0.692, *P* = 0.013) in the GO group. There were no statistically significant correlations between IgG4 and TRAb, TPOAb, or TgAb levels in any of the subgroup analyses. In all, 5 out of 12 patients from the moderate-to-severe GO group had active disease defined by the CAS ≥3. There was no statistically significant correlation between the CAS and IgG4 serum concentration in the GO group.

Patients with thyroid nodules had lower IgG4 levels than patients without thyroid nodules, but the difference was not statistically significant (35.7 [24.8; 41.53] mg/dL vs. 43 [30.1; 92.7] mg/dL, *P* = 0.064). The correlation between IgG4 levels and the number of thyroid nodules did not reach statistical significance (*ρ* = (–)0.263, *P* = 0.054). The IgG4 levels did not correlate with any other ultrasonographic parameters, including intrathyroidal blood flow, thyroid echogenicity, and thyroid volume (Table 3). We created a receiver operating characteristic (ROC) curve of IgG4 as a diagnostic tool for diagnosing moderate-to-severe GO (Figure 3). The optimal cut-off value determined by the highest Youden index was 49 mg/dL. At

Table 1. Comparison of clinical, biochemical, and ultrasonographic features between high IgG4 and normal IgG4 GD

	GD patients with IgG4 serum concentration within normal range (N = 54)	GD patients with high IgG4 serum concentration (N = 6)	P-value
IgG4 (mg/dL)	33.9 (25.73; 67.8)	159.5 (143.78; 174.93)	<0.001
Total IgG (g/L)	11.25 (10.31; 12.4)	11.39 (10.69; 14.58)	0.411
Age (years)	39.96 ± 13.44	38.83 ± 11.84	0.844
Gender (% of males)	14 (25.93%)	1 (16.67%)	1
TSH (μIU/mL)	0.01 (0.01; 1.33)	1.54 (0.34; 1.94)	0.095
ftT3 (pmol/L)	10.78 (4.74; 26.2)	6.06 (4.93; 6.76)	0.498
ftT4 (pmol/L)	30.16 (15.4; 53.4)	15 (13.18; 18.33)	0.112
TRAb (IU/l)	6.03 (2.56; 16.82)	2.68 (1.54; 3.56)	0.078
TPOAb (IU/mL)	122.5 (31.25; 281)	218 (154.5; 280.75)	0.538
TgAb (IU/mL)	71 (13; 325)	246 (84.5; 554.5)	0.204
CRP (mg/dL)	6.03 (2.56; 16.82)	2.68 (1.54; 3.56)	0.408
Creatinine (mg/mL)	0.57 (0.44; 0.73)	0.62 (0.57; 0.73)	0.267
ALT (U/l)	23.5 (17.25; 32)	24.5 (17.25; 28.75)	0.970
AST (U/l)	21.5 (16; 25)	22.5 (20.25; 26.25)	0.330
WBC (×10 ³ /μL)	6.46 ± 1.75	6.35 ± 2.3	0.878
RBC (×10 ⁶ /μL)	4.71 ± 0.4	4.76 ± 0.18	0.802
Hemoglobin (g/mL)	13.55 (12.63; 14.38)	14.1 (13.6; 14.38)	0.810
PLT (×10 ³ /μL)	4.71 ± 0.4	4.76 ± 0.18	0.802
Thyroid volume (cm ³)	29.82 ± 21.83	19.35 ± 15.25	0.233
Intrathyroidal blood flow	1 (0; 2)	1 (0.25; 1.75)	0.707
Thyroid echogenicity	1 (1; 2)	1 (1; 1.75)	0.792
Presence of any thyroid nodule (% of patients)	36.73	16.67	0.653
Presence of multiple (≥3) thyroid nodules (% of patients)	18.37	0	0.574
Moderate-to-severe GO (% of patients)	14.81	66.67	0.012

Continuous variables with a normal distribution are shown as mean ± SE, continuous variables without a normal distribution and ordinal variables are shown as median (lower quartile, upper quartile), and categorical variables are shown as percentage.

ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; ftT3, free triiodothyronine; ftT4, free thyroxine; GD, Graves' disease; GO, Graves' orbitopathy; IgG, immunoglobulin G; IgG4, immunoglobulin G4; PLT, platelets; RBC, red blood cell count; SE, standard error; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; TRAb, anti-TSH receptor antibodies; TSH, thyroid-stimulating hormone; WBC, white blood cell count.

Intrathyroidal blood flow was scored in the following way: 0—normal intrathyroidal blood flow, 1—mildly increased intrathyroidal blood flow, 2—severely increased intrathyroidal blood flow. Thyroid echogenicity was scored in the following way: 0—normal echogenicity, 1—slight hypoechogenicity, 2—deep echogenicity.

this cut-off value, the area under the curve (AUC) was 0.851 ($P < 0.001$), negative predictive value was 100%, positive predictive value 48%, sensitivity 100%, and specificity 73%.

4. Discussion

In our study, the IgG4 levels were higher in GO patients than in control subjects. However, the GD patients without GO did not differ significantly in IgG4 levels compared to healthy individuals. Previous studies have reported that IgG4 serum concentrations are higher in patients with GD than in healthy controls. Takeshima et al. (2014) compared serum IgG4 values of their group of patients with GD to the values of a healthy population reported previously by Yamamoto et al. (2012).

Other researchers compared IgG4 values between GD and self-recruited control groups (Bozkirli et al. 2015; Martin et al. 2017; Yu et al. 2017). However, most of the studies did not perform a separate analysis for GO and GD without GO patients. Only in one small study, the authors divided the GD group into GD with GO and GD without GO subgroups and compared the IgG4 serum concentrations of both subgroups with healthy controls (Bozkirli et al. 2015). Although other authors did not perform such subgroup analyses, the reported IgG4 serum concentrations of GD without GO patients and healthy controls were very similar (Takeshima et al. 2014; Yu et al. 2017), which is in concordance with our findings. Thus, the elevation of serum IgG4 levels should be considered as a finding predominately related to GO, not GD in general.

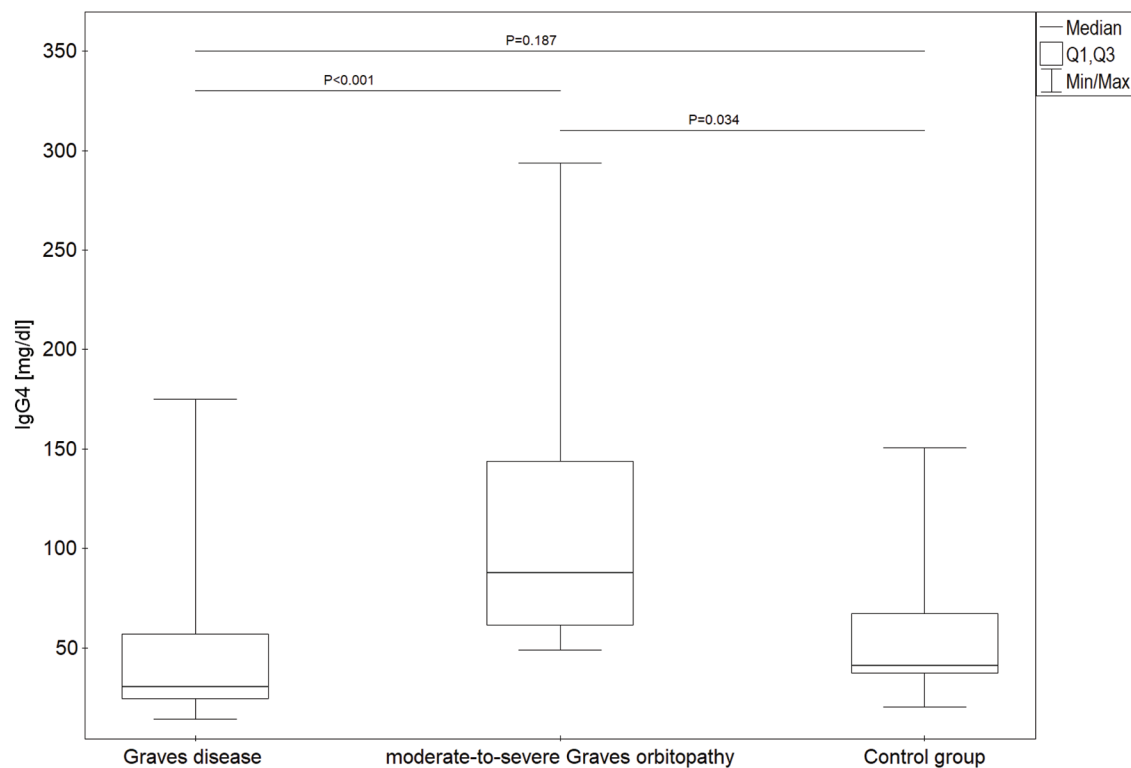


Fig 2. Boxplots comparing IgG4 serum concentration between GD without orbitopathy, moderate-to-severe GO, and the control group. GD, Graves' disease; GO, Graves' orbitopathy; IgG4, Immunoglobulin G4.

Table 2. Comparison of IgG, IgG4, and other biochemical features between hyperthyroid and euthyroid patients with GD

	Hyperthyroid GD (N = 32)	Euthyroid GD (N = 28)	P-value
IgG4 (mg/dL)	33 (23.78; 58.3)	52.4 (29.45; 95.6)	0.061
Total IgG (g/L)	11.72 (10.88; 13.09)	10.65 (9.77; 12.34)	0.098
TSH (μU/mL)	0.01 (0.01; 0.01)	1.55 (0.81; 2.25)	<0.001
FT3 (pmol/L)	25.72 (17.35; 31.95)	4.72 (4.44; 5.23)	<0.001
FT4 (pmol/L)	48.91 (38.08; 79.9)	15.14 (13.09; 16.01)	<0.001
TRAb (IU/l)	13.23 (6.43; 23.31)	2.09 (0.9; 3.98)	<0.001
TPOAb (IU/mL)	246 (59.75; 410.75)	63 (27; 147.25)	0.004
TgAb (IU/mL)	271 (35.25; 525.25)	20.5 (10.75; 164)	0.006
CRP (mg/dL)	1.15 (0.5; 2.53)	1.05 (0.48; 2.13)	0.953
Moderate-to-severe GO (% of patients)	9.38	32.14	0.050

Continuous variables with a normal distribution are shown as mean \pm SE, continuous variables without a normal distribution and ordinal variables are shown as median (lower quartile, upper quartile), categorical variables are shown as percentage. CRP, C-reactive protein; FT3, free triiodothyronine; FT4, free thyroxine; GD, Graves' disease; GO, Graves' orbitopathy; IgG, immunoglobulin G; IgG4, immunoglobulin G4; SE, standard error; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; TRAb, anti-TSH receptor antibodies; TSH, thyroid-stimulating hormone.

The prevalence of patients with high IgG4 GD in our cohort (10%) was similar to that reported in the recent systematic review on the role of IgG4 in GD (Olejars et al. 2021). The GD variant with elevated IgG4 serum concentration was more prevalent among patients with GO (33%) than patients without GO (4.2%). The higher prevalence of elevated IgG4 GD among GO patients was also reported by other authors and was as high as 37.5% in one Turkish study (Takeshima et al. 2014; Bozkirli et al. 2015; Yu et al. 2017). In two studies, the frequency of IgG4 serum concentration elevation was equal among GO and GD without GO patients. However, one of the authors used different criteria for defining high IgG4 GO (Martin et al. 2017), and the other one studied retrospectively a large cohort of GD patients who were qualified for radioiodine therapy (Comi et al. 2023), which was a significant selection bias, as patients with moderate-to-severe and active GO are typically not being referred for such a treatment. Some authors reported an association between IgG4 levels and the CAS (Bozkirli et al. 2015; Yu et al. 2017; Li et al. 2021), while others did not find such a relationship (Takeshima et al. 2014; Sy and Silkiss 2016). Other authors reported an association between the IgG4 levels and indices of active GO in magnetic resonance imaging of the orbits (Li et al. 2021; Olejars

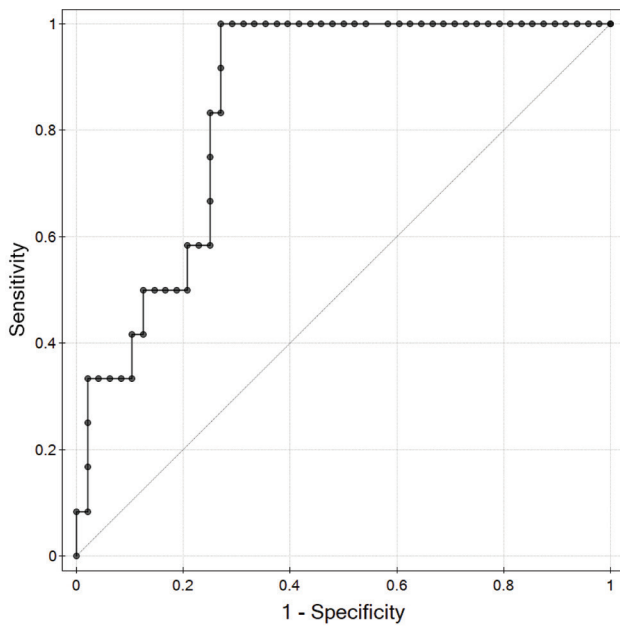


Fig 3. The ROC curve of IgG4 as a diagnostic tool for diagnosing moderate-to-severe GO. The optimal cut-off value determined by the highest Youden index was 49 mg/dL. At this cut-off value, the AUC was 0.851 ($P < 0.001$), negative predictive value was 100%, positive predictive value 48%, sensitivity 100%, and specificity 73%. AUC, area under the curve; GO, Graves' orbitopathy; IgG4, Immunoglobulin G4; ROC, receiver operating characteristic.

et al. 2023), while Luo et al. (2022) described bilateral muscle thickening in elevated IgG4 GO. Our study was not designed to check for detailed differences in ocular manifestations, as the number of GO patients was relatively low.

We compared the IgG4 serum concentration of patients with GD and untreated hyperthyroidism and patients with GD and euthyroidism after methimazole treatment. Patients with euthyroidism had slightly higher IgG4 levels, but the difference was not statistically significant. Similar observations were described in a study by Hiratsuka et al., in which IgG4 serum levels before and after methimazole treatment did not differ; however, this was based on an observation of only nine patients (Hiratsuka et al. 2020). Other authors found that patients with GD with elevated IgG4 serum concentrations tend to develop hypothyroidism after antithyroid treatment (Takeshima et al. 2014). As our study was not longitudinal in design, we were not able to assess time- and treatment-dependent changes in thyroid hormone levels. Martin et al. (2017) reported that patients with GD and elevated IgG4 serum concentration have lower triiodothyronine levels at diagnosis. In our cohort, the patients with high serum IgG4 levels did not differ from normal IgG4 GD patients in thyroid hormone levels. However, we found a negative correlation between IgG4 serum concentration and fT4 concentration.

Table 3. Correlations of selected biochemical, hormonal, anthropometric, and ultrasound parameters with serum IgG4 and IgG levels

	IgG4	Total IgG
IgG4	–	$\rho = 0.072, P = 0.586$
Total IgG	$\rho = 0.072, P = 0.586$	–
Age (years)	$\rho = (-)0.085, P = 0.517$	$R = (-)0.239, P = 0.066$
CRP	$\rho = (-)0.084, P = 0.523$	$\rho = (-)0.070, P = 0.594$
TSH	$\rho = 0.236, P = 0.070$	$\rho = (-)0.149, P = 0.258$
fT3	$\rho = (-)0.197, P = 0.131$	$\rho = 0.353, P = 0.006$
fT4	$\rho = (-)0.260, P = 0.045$	$\rho = 0.387, P = 0.002$
TRAb	$\rho = (-)0.270, P = 0.037$	$\rho = -0.032, P = 0.803$
TPOAb	$\rho = 0.052, P = 0.695$	$\rho = 0.130, P = 0.324$
TgAb	$\rho = 0.202, P = 0.685$	$\rho = 0.202, P = 0.122$
Creatinine	$\rho = 0.117, P = 0.373$	$\rho = 0.062, P = 0.637$
ALT	$\rho = (-)0.167, P = 0.202$	$\rho = 0.033, P = 0.800$
AST	$\rho = (-)0.047, P = 0.724$	$\rho = 0.103, P = 0.433$
WBC	$\rho = (-)0.0512, P = 0.697$	$R = (-)0.106, P = 0.421$
RBC	$\rho = 0.0852, P = 0.517$	$R = 0.219, P = 0.093$
Hemoglobin	$\rho = 0.033, P = 0.800$	$R = (-)0.133, P = 0.309$
PLT	$\rho = (-)0.033, R = 0.800$	$R = 0.142, P = 0.278$
Thyroid volume	$\rho = (-)0.233, P = 0.087$	$\rho = 0.145, P = 0.290$
Intrathyroidal blood flow	$\rho = (-)0.075, P = 0.594$	$\rho = 0.073, P = 0.603$
Thyroid echogenicity	$\rho = 0.077, P = 0.578$	$\rho = (-)0.153, P = 0.266$
Number of thyroid nodules	$\rho = (-)0.263, P = 0.054$	$\rho = (-)0.322, P = 0.117$

ALT, alanine transaminase; AST, aspartate transaminase; CRP, C-reactive protein; fT3, free triiodothyronine; fT4, free thyroxine; IgG, Immunoglobulin G; IgG4, Immunoglobulin G4; PLT, platelets; R, Pearson Correlation Coefficient; RBC, red blood cell count; TgAb, anti-thyroglobulin antibodies; TPOAb, anti-thyroid peroxidase antibodies; TRAb, anti-TSH receptor antibodies; TSH, thyroid-stimulating hormone; WBC, white blood cell count; ρ , Spearman's Rank Correlation Coefficient.

Bold font indicates statistically significant values.

The relationship between TRAb and IgG4 is still debated. Some of the studies conducted so far found a positive correlation between TRAb and IgG4 serum concentration (Yu et al. 2017; Hiratsuka et al. 2020; Luo et al. 2022), while others did not observe any association between those two parameters (Martin et al. 2017; Hiratsuka et al. 2020; Comi et al. 2023). Surprisingly, in our cohort, there was a negative association between IgG4 and TRAb serum concentrations. This could be explained by the fact that the shift toward IgG4 production usually occurs in the later stages of autoimmune disease after a prolonged time of antigen stimulation (Aalberse et al. 1983; Konecny et al. 2022). In our study, most patients with the highest IgG4 levels were GO patients with a longer-lasting disease and lower TRAb levels than patients with relatively new-onset GD. When

the groups with GO and GD were analyzed separately, this relationship was no longer observed.

Some authors reported a higher degree of thyroid hypoechogenicity in patients with elevated serum IgG4 GD (Takeshima et al. 2014; Torimoto et al. 2017). However, in our cohort, such an association was not found. Patients with thyroid nodules had lower IgG4 levels than those without thyroid nodules, but the difference was not statistically significant, with a borderline *P*-value of 0.064. There was also a negative correlation between the IgG4 serum concentration and the number of thyroid nodules, which did not reach statistical significance (*P* = 0.054). No association between IgG4 levels and thyroid nodules in GD has been reported so far. However, some data on the association between IgG4 antibodies and thyroid nodules is available from the HT population. IgG4-positive HT (diagnosed based on a histopathological examination) is associated with a higher risk of papillary thyroid cancer, bigger tumor size, and lymph node metastases (Yu et al. 2016). Similarly, in a cohort of patients from Egypt, serum IgG4 levels were found to be associated with thyroid nodule malignancy (Elshaer et al. 2022). In case of our patients, the observed nodules were usually unsuspecting and did not require fine needle biopsy. Even though the associations between IgG4 serum concentration and thyroid nodules were not significant in our cohort, the observed trends and borderline *P*-values warrant a study on a larger population.

Our research has shown that the IgG4 serum measurements could also be used as an adjunctive diagnostic tool for moderate-to-severe GO diagnosis, even at values much lower than the 135 mg/dL cut-off point used for IgG4-RD and IgG4-RTD. Serum IgG4 measurement can function as a rule-out test for GO. It has high sensitivity and negative predictive value. However, the positive predictive value was low; thus, it cannot be used as a diagnosis-confirming test. This is due to the fact that high serum IgG4 concentration is, in general, non-specific, as the elevation can be caused by a multitude of diseases, including infections, allergies, malignancy, and a variety of other respiratory, gastrointestinal, and rheumatic disorders. To avoid most of these factors, we applied strict exclusion criteria. In some people, a high IgG4 level can even be found without any tangible cause (Ebbo et al. 2012; Lang et al. 2016).

This study had several limitations. First, our center is known for being the only place in the region that treats GO with intravenous pulse therapy. Thus, patients with moderate-to-severe GO were referred preferentially to our in-patient center from a large area, while patients without GO were mainly selected from our outpatient clinic, which has a more local population. This might be a reason for a potential selection

bias. We did not control for a history of allergies, which are one of the factors that might influence IgG4 serum concentrations. However, patients with symptomatic allergies requiring treatment at the time of sampling were excluded. Our study was designed in a cross-sectional way. We compared distinct patient groups with different hormonal status. A study designed in a longitudinal way to observe hormonal and immunoglobulin changes during a certain period of time could have given different results.

5. Conclusions

The GD variant with high IgG4 serum concentration is associated with the higher occurrence of GO. IgG4 serum concentrations are higher in GO than in healthy controls. IgG4 serum concentration can be used as an adjunctive diagnostic tool in GD patients with suspected GO, mainly as a rule-out test due to its high sensitivity and negative predictive value. IgG4 serum levels are not related to total IgG serum concentration. Our study does not show a clear relationship between IgG4 serum concentration and thyroid hormones or antithyroid antibody levels. Patients with untreated hyperthyroidism and euthyroidism after methimazole treatment do not differ statistically significantly in IgG4 serum levels. Larger studies, especially with a longitudinal design and analyses of histopathological specimens, are still needed to better define and understand GD with elevated IgG4 serum concentration.

Declarations

Authors' Contributions

MO, ESP, and MR contributed to the conception and design of the study. MO, ESP, AK, and NSG recruited the patients. MO and AK organized the database. MO performed the statistical analysis. MO wrote the first draft of the manuscript. EW performed the laboratory analysis. All authors contributed to manuscript revision, read, and approved the submitted version.

Conflict of Interests Statement

Authors declare no competing interests.

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