Thyroid peroxidase antibody positivity and triiodothyronine levels are associated with pediatric Graves' ophthalmopathy

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Background: **Graves' ophthalmopathy (GO) occurs commonly in children with Graves' disease (GD). However, there are limited studies on the clinical manifestations and thyroid autoantibodies in pediatric GO. The aim of this study was to investigate the prevalence and risk factors of GO in childhood GD.**

Methods: **Clinical and biochemical data from children and adolescents with GD were retrospectively reviewed. Eighty patients under 19 years of age were included in the present study. We compared the clinical and biochemical differences between patients with and without GO.**

Results: **Thirty-nine percent of the patients had GO, and 81% of the GO patients were females. Of these, two patients showed unilateral GO. Triiodothyronine (T3) levels were higher in GO patients than in those without GO. Anti-thyroglobulin antibody and thyroid stimulating hormone receptor antibody titers were not significantly different between the two groups. Anti-thyroid peroxidase antibody (TPO Ab) positivity was 68% in the patients with GO and only 47% in the patients without GO. In multivariate regression analysis, high T3 levels and TPO Ab positivity were related to the presence of GO.**

Conclusion: **In children and adolescents with GD, TPO Ab positivity and high T3 levels could act as predictive factors for the presence of GO.**

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Introduction

Graves' disease (GD) is the most common cause of thyrotoxicosis in the pediatric population. Affected children suffer from abnormal physical growth, impaired school performance, and complain of cosmetic problems characterized by ocular manifestations, so-called Graves' ophthalmopathy (GO). According to a recent study,[1] GO occurs in 33% of the children and adolescents with GD. In adults, GO is characterized by increased retrobulbar tissue, enlargement of the extraocular muscles, and elevation of intraocular pressure, leading to eyelid swelling, proptosis, and even loss of vision.[1,2] GO is less severe in children, with the predominant eye manifestations being exophthalmos and involvement of the soft tissues; eye muscles and optic function are infrequently affected.[2-5] Nevertheless, GO can still significantly impair the quality of life of affected children. For instance, prominent exophthalmos can be very stressful to adolescents who are conscious of their appearance. Furthermore, severe GO can have life-long effects on the quality of life of the patients.[6]

GO often develops concomitantly with hyperthyroidism, but it could precede or follow hyperthyroidism. GO is usually bilateral, but it can be asymmetric or unilateral.[7] Case series report that even in the absence of clinical manifestations, imaging studies could reveal subtle orbital changes in most GD patients. Common ocular symptoms, including diplopia and symptoms related to corneal exposure, such as photophobia, tearing, grittiness, and pain, may interfere with daily activities.[7] Bartley et al[8] indicated that the incidences of eyelid retraction, exophthalmos, extraocular muscle dysfunction, ocular pain, and lacrimation were 91%, 62%, 43%, 30%, and 23%, respectively. Studies[9,10] on GO pathogenesis in adults have revealed an association

between thyroid stimulating hormone receptor antibodies (TSHR Ab) and the occurrence and severity of GO. GO is more prevalent in female patients, whereas severe GO is more common in male patients.[11] Some studies[12] showed that patients treated with radioactive iodine were more prone to GO than those treated with antithyroid drugs. Few studies[2,13,14] have focused on the clinical and laboratory factors that affect the pathogenesis of GO in children. The present study aimed to identify the predictive factors associated with GO in children in comparing the clinical features of GD patients with and without GO.

Methods

Patients

This study included all GD patients who were diagnosed at age 18 or younger and were followed up for more than one year at St. Vincent Hospital of the Catholic University of Korea, Suwon, South Korea from January 2000 to December 2012. A total of 80 patients (17 boys and 63 girls) met these criteria. Diagnostic criteria for GD were defined as low serum thyroid stimulating hormone (TSH) levels with high serum triiodothyronine (T3) and free thyroxin (fT4) levels and positivity for serum TSHR Ab. The diagnosis of GO was according to Vision, Inflammation, Strabismus, and Appearance (VISA) classification[15] based on the presence of clinically apparent symptoms and signs of disease, including visual acuity loss, eyelid edema, eyelid retraction, exophthalmos, diplopia, photophobia, tearing grittiness, ocular pain, lacrimation and impaired motility due to ocular myopathy. Exophthalmos is diagnosed by measurement with a Hertel exophthalmometer also according to the VISA classification. However, there are no diagnostic criteria for young children with exophthalmos, children less than 10 years of age were diagnosed with exophthalmos by description of the patients and their parents, and confirmed by one ophthalmologist.

Orbital imaging with the use of computed tomography (CT) was warranted to GD patients in whom ophthalmopathy is unilateral. The findings of orbital imaging in patients with GO included enlargement of the extraocular muscles, an increase in intraorbital fat tissue, or both.[16] This study complied with the recommendations of the *Declaration of Helsinki* and was approved by the Institutional Review Board of the St. Vincent Hospital of the Catholic University of Korea.

Laboratory methods

The levels of serum T3, fT4, and TSH were measured by a chemiluminescent microparticle immunoassay using an Architect i2000 analyzer (Abbott, Abbott Park, IL, USA). The levels of serum anti-thyroid peroxidase antibody (TPO Ab) and anti-thyroglobulin antibody (Tg Ab) were measured by a chemiluminescent immunometric assay using an Immulite 200 analyzer (Siemens Healthcare Diagnostic Products Ltd., Llanberis, Gwynedd, UK). TSHR Ab levels, considered to reflect thyrotropin binding inhibitory immunoglobulin (TBII) levels, were measured by an electrochemiluminescence immunoassay using a Cobase 411 analyzer (Roche Diagnostics GmbH, Mannheim, Germany). Reference values were as follows: T3, 0.58-1.9 ng/mL; fT4, 0.7-1.79 ng/dL; TSH, 0.35-4.95 μIU/mL; TPO Ab, <30 IU/mL; Tg Ab, <40 IU/mL; and TSHR Ab, <1.75 IU/L.

Statistical analysis

Statistical analyses were performed using SPSS for Windows (version 12.0, SPSS Inc., Chicago, IL, USA). Continuous variables were expressed as the mean±standard deviation of values. Student's *t* test and the Mann-Whitney *U* test were used for the analysis of continuous data and the chi-square test or Fisher's exact test was used for the analysis of categorical variables. Multivariate logistic regression analysis was performed to investigate the odds ratios (OR) and 95% confidence interval (CI) of clinical predictors among several parameters associated with the presence of GO. *P* values less than 0.05 were considered statistically significant.

Results

Clinical characteristics of patients with GD

The medical records of 98 patients who met the eligibility criteria were retrospectively reviewed. Eighteen of these patients were excluded from the study, as they were referred to our clinic in the middle of their treatment or had incomplete medical records. Thus, a total of 80 patients (17 boys and 63 girls) who were newly diagnosed and were followed up for more than 12 months at St. Vincent Hospital were included in the study. The mean age of the patients was 12.2±3.5 years (range, 2-18 years). Of the 80 patients, 31 (39%) had GO (Table 2). Most of the patients had cardiac manifestations (73%), goitre (65%), and weight loss (60%). Symptoms present in a minority of patients included restlessness, enuresis, and diarrhea; one patient showed weight gain (Table 1). Table 1 illustrates the clinical manifestations of GD in all patients. Orbit CT scan was performed in 2 patients with unilateral GO and revealed rectus muscle hypertrophy rather than lipid hyperplasia. All patients were non-smokers. The patients with GO had exophthalmos and eyelid

Table 1. Clinical manifestations of patients with Graves' disease

Clinical manifestations	*n* (%)
Cardiac manifestations	58 (73)
Goiter	52 (65)
Weight loss	48 (60)
Heat intolerance	45 (56)
Ophthalmopathy	31 (39)
Restlessness	25 (31)
Enuresis	3 (4)
Diarrhea	3 (4)
Hyperglycemia	1 (1)
Weight gain	1 (1)
Total	80 (100)

Table 2. Comparison of clinical and laboratory findings between patients with Graves' ophthalmopathy and without Graves' ophthalmopathy

Parameters	GO	Without GO	*P* value
Age at diagnosis (y)	11.29±3.60	12.76±3.27	0.523
Sex, *n* (%)			
Male (*n*=17)	6 (19%)	11 (22%)	0.787
Female (*n*=63)	25 (81%)	38 (78%)	
Goiter, *n* (%)			
Present	23 (74%)	29 (59%)	0.230
Absent	8 (26%)	20 (41%)	
Weight loss, *n* (%)			
Present	25 (81%)	23 (47%)	0.005
Absent	6 (19%)	26 (53%)	
Cardiac manifestations, *n* (%)			
Present	24 (77%)	34 (69%)	0.608
Absent	7 (23%)	15 (31%)	
Heat intolerance, *n* (%)			
Present	19 (61%)	26 (53%)	0.643
Absent	12 (39%)	22 (47%)	
TPO Ab, *n* (%)			
Present	21 (68%)	22 (45%)	0.066
Absent	10 (32%)	27 (55%)	
TPO Ab titer (IU/mL)	328.08±399.18	277.53±420.00	0.518
Follow-up	381.30±382.23	416.83±440.13	0.791
Tg Ab, *n* (%)			
Present	16 (52%)	26 (53%)	1.000
Absent	15 (48%)	23 (47%)	
TBII titer (IU/L)	32.470±38.850	19.500±21.470	0.142
T3 (ng/mL)	5.401±2.457	4.158±1.969	0.006
fT4 (ng/dL)	3.726±1.298	3.291±1.398	0.537
TSH (μIU/mL)	0.002±0.005	0.025±0.127	0.067
Total (*n*=80)	31 (39%)	49 (61%)	

GO: Graves' ophthalmopathy; TPO Ab: anti-thyroid peroxidase antibody; Tg Ab: anti-thyroglobulin antibody; TBII: thyrotropin binding inhibitory immunoglobulin; T3: triidothyronine; fT4: free thyroxine; TSH: thyroid stimulating hormone.

Table 3. Factors associated with Graves' ophthalmopathy

Parameters	Odds ratio	95% CI	*P* value
Age	0.916	0.794-1.056	0.226
Sex	0.954	0.287-3.177	0.939
T3 (ng/mL)	1.291	1.046-1.593	0.017
fT4 (ng/dL)	1.267	0.905-1.774	0.168
TPO Ab	2.577	1.007-6.599	0.048
Tg Ab	1.060	0.431-2.608	0.899
TBII (IU/L)	1.017	0.997-1.036	0.095

CI: confidence interval; T3: triidothyronine; fT4: free thyroxine; TPO Ab: anti-thyroid peroxidase antibody; Tg Ab: anti-thyroglobulin antibody; TBII: thyrotropin binding inhibitory immunoglobulin.

retraction, and one patient with bilateral exophthalmos had conjunctival injection. Only one patient with unilateral GO had diplopia and underwent medical treatment in the ophthalmologic department.

Clinical profiles of GD patients with and without GO

The patients were divided into two groups: GD patients with GO in the group 1 and GD patients without GO in the group 2. The mean ages of patients in the groups 1 and 2 were 11.29±3.60 and 12.76±3.27 years, respectively. The proportion of female patients was slightly higher in the group 1 (81% *vs.* 78%). Goitre was present in 74% of the patients in the group 1 and 59% of the patients in the group 2. The proportion of patients showing weight loss in the group 1 was significantly higher than that in the group 2 (81% *vs.* 47%, *P*=0.005). Clinical manifestations such as heat intolerance or cardiac symptoms were not significantly different between the two groups (Table 2).

Biochemical profiles of GD subjects with and without GO

The serum levels of T3, fT4, TSH, TPO Ab, Tg Ab, and TBII were measured in all participating patients (Table 2). The mean serum T3 level was 5.40±2.46 ng/mL in the group 1, which was significantly higher than that in the group 2 (*P*=0.006). Otherwise, there was no significant difference in the mean serum levels of fT4 and TSH between the two groups. TPO Ab was present in 68% of the patients in the group 1 and only 47% of the patients in the group 2 (*P*=0.066). Tg Ab was present in 52% and 53% of the patients in groups 1 and 2 respectively, with no significant difference. The TBII titre was higher in the group 1 (32.47±38.85 μIU/mL) than in the group 2 (19.50±21.47 μIU/mL), although the difference was not statistically significant. Follow-up titres of TPO Ab at euthyroid state after a treatment in the group 1 were not significantly different from those at initial diagnosis. These findings were also found in the group 2 (277.53±420.00 IU/mL *vs.* 416.83±440.13 IU/mL, *P*=0.212). There were no significant differences between GO patients with TPO Ab and without TPO Ab in terms of orbital symptoms and signs. The two patients with conjunctival injection and diplopia were negative for TPO Ab.

Logistic regression analysis of clinical and biochemical profiles associated with GO

Table 3 summarizes the associations between biological factors and GO in children. Using multivariate logistic regression analysis, we found significant association between the presence of GO and the serum levels of T3 (*P*<0.05; OR=1.291; 95% CI=1.046-1.593) and TPO Ab positivity (*P*<0.05; OR=2.577; 95% CI=1.007-

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6.599) in children with GD (Table 3). TBII levels were not significantly associated with the presence of GO. Age and sex were not significantly related to GO.

Discussion

In this study, we found that high levels of T3 and TPO Ab positivity were associated with the presence of GO in childhood GD. High T3 levels suggest the severity of hyperthyroidism that could directly cause GO in childhood GD. In this study, weight loss was significantly different between the two groups. This finding could reaffirm that GD severity is closely associated with the development of GO. Using logistic regression analysis, we identified that the positivity of TPO Ab was independently related to the presence of GO. However, TPO Ab titers were not significantly different between the two groups although the titers were slightly high in the GO group. Also, follow-up TPO Ab titers after acquiring euthyroid state were not significantly different compared to the initial titers. The role of TPO Ab in autoimmune thyroid disease remains controversial. Previous studies[17-21] showed TPO Ab in the serum of adult patients with GO. Wright-Pascoe et al[19] proposed that the presence of both TPO Ab and Tg Ab was correlated with the incidence of adulthood GO. Hence, TPO Ab is considered to cause inflammatory destruction of thyroid cells and induce the release of thyroid hormone into the blood stream during the early phase of destruction. In contrast, two different studies[20,21] demonstrated that TPO Ab negativity was associated with an increased risk of GO in adults. These studies assessed both adult and adolescent patients, in contrast to our study, which was limited to pediatric patients with GD.

The interaction of genetic and environmental factors plays a key role in GD.[22] Smoking is a well-known environmental risk factor for the development and aggravation of GO, and passive smoking is now being considered an important cause of GO in children.[1] Krassas et al[1] identified that a higher proportion of children below 10 years old develop GO in countries with a higher prevalence of smoking (25%). Countries with a lower prevalence of smoking also have a lower incidence of GO in children aged less than 10 years (19%).[1] Because of the retrospective nature of this study, although our study patients were not active smokers, we were unable to assess the effect, if any, of passive smoking on the presence of GO. In our study, we found that GD pediatric patients with GO had a higher titre of TSHR Abs (TBII) than those without GO, although this difference was not statistically significant. Many reports[9,10,14] have shown that TBII is related to the presence and severity of GO. Recently two methods are used to measure TSHR antibody levels. The TBII assay measures the levels of immunoglobulins that inhibit binding of TSH to TSHR: as such, this assay measures the levels of both thyroid-stimulating immunoglobulins (TSIs) and thyroid-blocking immunoglobulins (TBIs) that target the TSH receptor.[23] The second method is a bioassay that can distinguish between TSI and TBI through their effects on cyclic AMP production.[24] It was reported that TSIs are more significantly associated with GO than TBII.[9] However, in our study, we did not distinguish between TSIs and TBIs.

In the present study, the prevalence of GO (39%) in GD patients was similar to that reported previously, ranging from 25% to 50%.[16,25-28] In the previous studies, there was female predominance in GO patients, with a girl to boy ratio of 4:1. However, other reports suggested that the female to male ratio is likely to be 2:1, and men with GD appear to have a more severe form of GO.[11] Compared to its severity in adults, GO in children is milder, and most children could improve after becoming euthyroid. Although the exact mechanisms involved in the spontaneous resolution of GO in children have not been established, physiologic decompression caused by increased orbital volume due to childhood somatic growth is thought to be involved in the process, especially in patients diagnosed at a prepubertal age.[12] Therefore, a wait-and-see policy is the recommended treatment for GO in children.[2] However, if GO is present or worsening even after the patient shows normal results in thyroid tests, steroid treatment may be recommended.[2] In the present study, orbital symptoms did not improve in two girls after they acquired an euthyroid state. One was diagnosed at 14 years of age with severe unilateral GO and diplopia and treated with steroids by an ophthalmologist. The other, diagnosed at 10 years of age, had bilateral exophthalmos and lid retraction without other symptoms. She was therefore followed up closely.

This study has limitations. It has a small number of patients and is of cross-sectional nature. Moreover, we only evaluated the clinical parameters of GD patients.

In conclusion, the present study suggests that the positivity of TPO Ab could act as a predictive factor for the presence of GO in children although clinical and radiologic evidences are lacking for its diagnosis.

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