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Levels of Antithyroid Antiperoxidase and Antithyroglobulin Antibodies in Patients with Graves Hyperthyroidism – Predictors of Initial and Sustained Remission and Consecutive Hypothyroidism

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Abstract

BACKGROUND: Initial and sustained remission is the principal objective in patients with Graves hyperthyroidism (GH) treated with antithyroid drugs (ATD).

AIMS: Our study aimed to investigate the predictive value of antithyroid antiperoxidase (aTPO) and antithyroglobulin (aTg) levels on initial and sustained remission or consecutive hypothyroidism in subjects with GH treated with ATD.

METHODS: Randomized, prospective, and longitudinal study during period 2013–2018 was performed. Eighty GH patients (thyrotropin [TSH] <0.01 mIU/L, free thyroxine [FT4] >30 pmol/L, triiodothyronine [FT3] >8 pmol/L), 59 females (73.75%), and 21 males (26.25%), aged 51 ± 12 years were included in the study. Subjects were divided into four subgroups (each with 20 patients) according to aTPO and aTg levels: Group I – no antibodies; Group II – (<500 IU/mL), Group III – (500–1000 IU/mL), and Group IV – (>1000 IU/mL). All subjects underwent 24 months of propylthiouracil treatment with follow-up of 24 months after remission (TSH >0.4 mIU/L, FT4 11–25 pmol/L, FT3 2.8–6.5 pmol/L). Blood samples were analyzed every 4 months.

RESULTS: In Group I, 11 (55%) of the patients attained remission; during follow-up, 5 (45%) of them had disease relapse. In Group II, 12 (60%) attained remission and 5 (42%) had relapse. In Group III, 15 (75%) attained remission, 2 (13%) had disease relapse, and 4 (26%) developed hypothyroidism. In Group IV, 18 (90%) attained remission, 1 (5%) had disease relapse, and 9 (50%) developed hypothyroidism. Patients with values below 500 IU/mL attained remission in 60%, similar as the group without antibodies in 55% ($p < 0.3$). Patients in Group III and IV had significantly higher remission rates compared to Group I and II ($p < 0.001$). Baseline values of aTPO and aTg >1000 IU/mL were significant predictors of consecutive hypothyroidism ($p < 0.05$).

CONCLUSION: Baseline aTPO and aTg values above 500 IU/mL were significantly prognostic for attaining and sustaining remission in GH and values above 1000 IU/mL were significant predictors of consecutive hypothyroidism.

Introduction

Graves' disease is an organ-specific autoimmune thyroid disorder. It is the most frequent type of hyperthyroidism in iodine-sufficient countries, involving 2 - 5% of the European population [1]. Etiopathogenesis of this disorder is still not entirely understood, but clinical experiences revealed very often familial occurrence of different autoimmune thyroid disorders (Graves' and Hashimoto thyroiditis) among close relatives, as well as variations in the presence and titers of different anti-thyroid antibodies (ATAb) in patient's blood during disease course. This is indicating that maybe Graves' and Hashimoto's thyroiditis are two spectrums of the thyroid autoimmune disorder and that development to one or another state is probably conducted by a specific set of environmental factors, in conformity with genetic susceptibility [2], [3]. The identified autoimmune thyroid disease susceptibility genes include immune-modulating genes, such as the major histocompatibility complex, cytotoxic T-lymphocyte antigen-4, CD40 molecule, protein tyrosine phosphatase-22, thyrotropin (TSH) receptor and thyroglobulin [4]. Diagnosis often includes, besides clinical examination of the patient, thyroid gland ultrasound, also laboratory tests, such as analysis of thyroid hormones and ATAb, or thyroid stimulating hormone receptor antibodies (TRAb), antithyroid peroxidase (aTPO) antibodies, and antithyroglobulin (aTG) antibodies. TRAbs are specific biomarkers for Graves' hyperthyroidism (GH). Most immunoassays evaluate the presence or absence of TRAb, but not their functional status, meaning evaluation of their stimulatory or blocking effects [5]. Furthermore until now, it is not clear the relation between the presence of the other types of antibodies (aTPO and aTG) and the disease course. Initial and sustained remission is the principal objective in patients with GH treated with anti-thyroid drugs (ATD). Our study aimed to investigate the

predictive value of aTPO and aTg levels on initial and sustained remission or consecutive hypothyroidism in subjects with GH treated with ATD.

Materials and Methods

Randomized, prospective and longitudinal study during period 2013–2018 was performed. Eighty GH patients were included with inclusion criteria: initial free thyroxine (FT4) value above 30 pmol/L, TSH value below 0.01 mIU/L, and free thriiodothyronine (FT3) above 8 pmol/L. According to gender distribution, 59 were females (73.75%) and 21 males (26.25%) (Figure 1), aged 51 ± 12 years. In all patients, ultrasound examination (US) of the neck (linear transducer 7.5–10 MHz) was performed as well as thyroid scan after 15–20 min of intravenous application of pertechnetate. Patients with autonomous toxic adenomas and toxic nodular goiters were excluded from the study. In all patients' blood samples were drawn and from laboratory tests we analyzed TSH, FT4, FT3, aTPO, aTg, with Immulite 2000 Immunoassay test. Samples were analyzed every 4 months during a 24 months interval. Subjects were divided into four subgroups (each with 20 patients) according to aTPO and aTg levels: Group I – no antibodies; Group II – (<500 IU/mL), Group III – (500–1000 IU/mL), and Group IV – (>1000 IU/mL). All subjects underwent 24 months of propylthiouracil or thiamazole treatment with follow-up of 24 months after remission, confirmed when laboratory values were TSH >0.4 mIU/L, FT4 11–25 pmol/L, and FT3 2.8–6.5 pmol/L. The data were statistically analyzed in the SPSS software package, version 22.0 for Windows (SPSS, Chicago, IL, USA).

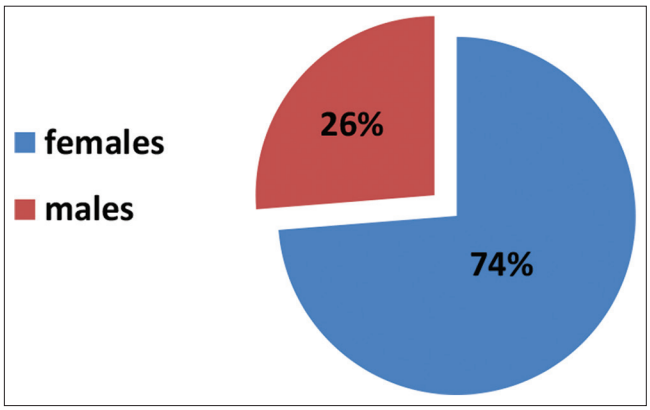

Figure 1: Gender distribution in all patients showed predominantly female gender representation among patients with hyperthyroidism

Results

In Group I, 11 (55%) of the patients attained remission, during follow-up, 5 (45%) of them had disease relapse. In Group II, 12 (60%) attained remission, and 5 (42%) had relapse. In Group III, 15 (75%) attained remission, 2 (13%) had disease relapse, and 4 (26%) developed hypothyroidism. In Group IV, 18 (90%) attained remission, 1 (5%) had disease relapse, and 9 (50%) developed hypothyroidism.

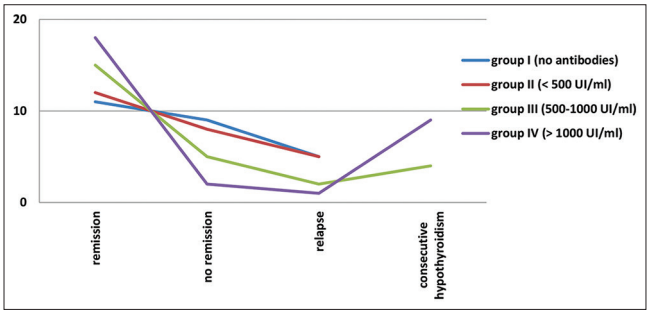

Figure 2: Correlation between antibody levels, remission, and consecutive hypothyroidism

Patients with values for aTPO below 500 IU/mL attained remission in 60%, similar as the group without antibodies in 55% ($p < 0.3$). Patients in Group III and IV had significantly higher remission rate compared to Group I and II ($p < 0.001$). Baseline values of aTPO and aTg>1000 IU/mL were significant predictors of consecutive hypothyroidism ($p < 0.05$) (Figures 2 and 3).

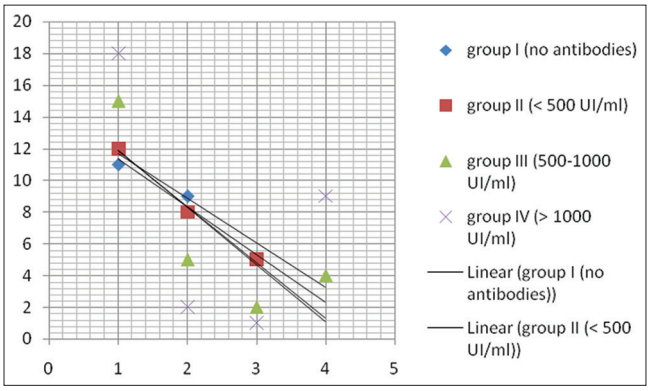

Figure 3: Cutoff value of thyroid antibodies in correlation with remission and consecutive hypothyroidism

Discussion

The incidence of autoimmune thyroid disease was evaluated by McGrogan *et al*., reviewing the database of Medline, EMBASE, and Science Direct from the past two decades. Their results reported an incidence of autoimmune hypothyroidism that varied between 2.2/100 000/year (males) and 498.4/100 000/year (females) and for autoimmune hyperthyroidism, incidence ranged from 0.70/100 000/year (Black males) to 99/100 000/year (Caucasian females) [6].

Routinely thyroid autoantibodies are not checked unless some thyroid abnormality is previously detected. Although TRAbs (TSHRAb, also known as

TRAb) are thyroid antibodies detected in autoimmune GH for prediction of the severity of the disease and prediction of disease recurrence, the other two antibodies, aTPO and aTG, are also evaluated as an early predictors of the disease course and the development of a spontaneous hypothyroidism. Our study revealed that patients with higher values for aTPO and aTg had significantly higher remission rate, compared to patients in the two groups with lower values for indicated ATAb and also we found that baseline values of aTPO and aTg >1000 IU/mL were significant predictors of a consecutive hypothyroidism ($p < 0.05$). It is postulated that autoimmune thyroid disorders are response of thyroid tissue damage that is predisposed by aTPO, either mediated by the complement pathway or by antibody-dependent cell-mediated cytotoxicity. By this way, thyroid cells release pro-inflammatory molecules that create an inflammatory reaction and lead to tissue destruction [7].

Other studies described that the presence of aTPO in GH patients resulted in a more aggressive disease pattern with higher relapse rate, mainly in females and younger age groups [8]. The underlying mechanism of the GH is T lymphocyte thyroid infiltration with B lymphocyte activation that leads to immune intolerance, as well as an increase in the synthesis and secretion of autoantibodies directed against the TSH receptor. Consequently, this interaction results in goiter, hyperthyroidism, ophthalmopathy, and dermopathy [9]. The human leukocyte antigen (HLA) system is responsible for binding to a specific antigen and further initiation of the immune response, mediated by autoreactive TLs (CD4+/CD8+), as well as for cytokine synthesis and secretion. In GH currently, HLA-DR3 dominates with 40–50% of GH patients in contrast to 15–30% of the general population [10]. In GH, an increase in the expression of CD152 has been found in TLs, suggesting the defective function of CD152. Different threonine/alanine transitions in the *CTLA4* gene were found in GH patients that lead to errors in its functioning in the endoplasmic reticulum, causing an inefficient glycosylation reaction, and a reduced expression of CTLA4 on the surfaces of TLs, decreasing their inhibitory function [11].

TRAbs are the main hallmark in GH and increase values of TRAb are found in 90% of GH patients, but also their presence is detected in up to 20% of Hashimoto thyroiditis, and in 10–75% of atrophic thyroiditis patients [12]. TRAb according to their effect can be stimulating and blocking. Stimulating antibodies are oligoclonal and belong to IgG1 class, while blocking antibodies are polyclonal and not restricted to a specific subclass [13].

aTPO antibodies can activate complement, destroy thyrocytes, and act as competitive inhibitors of enzymatic activity [14]. These antibodies can be of any class of IgG, although some studies indicated a higher prevalence of IgG1 (70%) and IgG4 (66.1%) compared to IgG2 (35.1%) and IgG3 (19.6%) [15].

Caturegli *et al*. analyzed the IgG subclass of aTg antibodies and they indicate that aTg antibodies do not fix complement because the epitopes are too widely spaced to allow cross-linking and also they point out that aTg antibodies in GH belong mainly to the IgG4 class, which is not complement binding [16]. The prevalence of aTPO and aTg antibodies is high in patients with GH and HT, while TRAb antibodies are common in GH patients but relatively rare in patients with HT [13].

Siriwardhane *et al*. in their study analyzed if the early appearance of thyroid autoantibodies in euthyroid subjects was a predictor for later development into hypothyroidism and hyperthyroidism. Their study revealed that aTPO autoantibodies were more sensitive in predicting further subclinical/overt hypothyroidism and hyperthyroidism development and that it may be beneficial to consider testing for aTPO in conjunction with the primary thyroid markers, TSH and FT4 [17].

Azizi *et al*. found that patients with positive aTPO were associated with high baseline free T4, while not with thyroid eye disease [18]. Similar results were demonstrated by Alhubaish *et al*. patients with aTPO antibodies versus those with negative antibodies showed higher pretreatment-FT4 level (3.7 ± 0.2 vs. 3.0 ± 0.2 with $p = 0.021$), baseline TRAb level of more than 6.4 IU/mL, and giraffe appearance on thyroid ultrasound [19].

Conclusion

Our study revealed that baseline aTPO and aTg values above 500 IU/ml were significant prognostic factors for attaining and sustaining remission in GH and values above 1000 IU/ml were significant predictors of consecutive hypothyroidism. This funding supports the need to evaluate baseline aTPO an aTg values in therapeutic management of GH patients as an early predictors of the disease course and the development of a spontaneous hypothyroidism. Further research including larger number of subjects and also longer follow up period could improve understanding the real clinical value of thyroid antibodies in follow up of GH patients.

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