CLINICAL SIGNIFICANCE OF AUTOANTIBODIES TO PARIETAL CELLS IN PATIENTS WITH AUTOIMMUNE THYROID DISEASES

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ABSTRACT
Clinical significance of autoantibodies to parietal cells (PCA) in patients with autoimmune thyroid diseases (ATD) remains contradictory.
AIM: To characterize the frequency of PCA in patients with ATD; to clarify the role of gender and age in PCA positivity; to analyze the association of PCA with gastric or hematologic symptoms and with the levothyroxine dose required to achieve a serum TSH level within the normal range in treated Hashimoto’s thyroiditis (HT) patients.
PATIENTS AND METHODS: PCA were measured using ELISA in 137 HT patients, divided into tree subgroups according to the thyroid function: group I included subjects with normal thyroid function; group II included patients with hypothyroidism, in group III were enrolled subjects treated with levothyroxine (LT4). We also studied the PCA positivity in 14 patients with active Graves’ disease and in 23 healthy controls.
RESULTS: PCA positivity was found in 51 patients with autoimmune thyroid diseases, with an overall prevalence of 33.8%. No significant differences in PCA were observed between the groups of HT patients. The frequency of PCA in both genders was similar and there were no difference depending on age. In 7.3% of HT patients in different stages of disease we found clinically relevant gastric and/or hematologic symptoms; in 70% of them PCA were positive (OR = 5.5; 95% CI: 1.2 – 28.4; p = 0.009). PCA positive hypothyroid HT patients required higher LT4 doses than PCA negative (1.46 μg/kg vs 1.24 μg/kg, p = 0.04).
CONCLUSIONS: PCA may be present in different stages in HT patients; this does not depend on the severity of disease. PCA concentrations may predict symptoms of atrophic gastritis in patients with Hashimoto’s thyroiditis. PCA positive HT patients require higher replacement doses of LT4. Presence of anemia, particularly microcytic anemia, was suggestive of undiagnosed atrophic gastritis.

Key words: parietal cell antibodies, Hashimoto’s thyroiditis

INTRODUCTION
Autoimmune thyroid diseases (ATD), including Hashimoto’s thyroiditis (HT) and Graves’ disease (GD) are organ-specific autoimmune disorders defined by lymphocytic infiltration of the thyroid.1,2 HT is the most common underlying cause of hypothyroidism. This disorder is most commonly found in middle-aged and elderly females, but it also occurs in other age groups.3 The severity of HT vary among patients. Most patients with HT maintain a lifetime euthyroid state without any medical treatment, whereas others become hypothyroid. ATD are often accompanied by other autoimmune conditions such as autoimmune gastritis and pernicious anaemia.4,5
Autoimmune gastritis is characterized by atrophy of the corpus and fundus and the presence of circulating autoantibodies to the parietal cells (PCA). PCA are seen in atrophic gastritis (AG) and pernicious anaemia. Chronic autoaggression to the gastric proton pump, H+/K+ ATPase, may result in decreased gastric acid secretion, hypergastrinemia and iron deficiency anemia.6,7 Clinical significance of PCA in patients with autoimmune HT remains contradictory. A weaker but still significant correlation has been found between these gastric disorders and severity of autoimmunity of Hashimoto’s thyroiditis in a study of Garcia et al.8 The frequency of PCA in different stages of activity of HT and their possible impact on the health of the patient remains unclear.

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In this respect, the present study aimed to characterize the frequency of PCA in 137 patients with HT in different stages of disease activity and 14 patients with active Graves’ disease; to clarify the role of gender and age in PCA positivity; to analyze the association of PCA with gastric or hematologic symptoms and with the levothyroxine dose required to achieve a serum TSH level within the normal range in treated HT patients.

PATIENTS AND METHODS
We recruited in a prospective study 137 outpatients with Hashimoto’s thyroiditis (mean age 49.0 ± 1.2 yrs; range 12–74 yrs; 8 (6% ± 2.0) men and 129 (94% ± 2.0) women) from the Department of Internal Medicine, Stara Zagora University Hospital (Bulgaria). The diagnosis for all patients was made on the basis of enlarged thyroid glands, elevated TPO Abs and/or Tg Abs and the typical hypoechogenicity of the thyroid in high-resolution sonography. In negative TPO Abs patients fine needle aspiration biopsy was performed finding the typical cytological features of autoimmune thyroiditis. The patients were divided into three subgroups according to their thyroid function. Group I (n = 41) included subjects with normal thyroid function (TSH and fT4 within the normal range). Group II (n = 25) included patients with hypothyroidism (high levels of TSH and low or normal serum levels of fT4). In group III (n = 71) we included subjects with hypothyroidism treated with levothyroxine (LT4) in a dose sufficient to maintain TSH and fT4 within the normal range. Levothyroxine was administered in the fasting state, mean levothyroxine doses were 88.3 ± 30.6 mg daily (range, 25-200 mg).

We studied also 14 patients (mean age 51.0 ± 4.2 yrs; range 19–68 yrs; 1 (7% ± 6.8) man and 13 (93% ± 6.8) women with active Graves’ disease. Blood samples obtained from 23 healthy women (mean age 49.0 ± 2.4 yrs; range 20–67 yrs) with no family history of autoimmune disease were used as controls.

We excluded from HT, GD patients and healthy controls, all smoking and alcohol drinking subjects, as well as individuals suffering from acute or chronic diseases.

Informed consent was obtained from all participants in the study in accordance with the ethical guidelines in the Helsinki Declaration.

Fasting venous blood samples were collected between 8:00 and 10:00 am. Serum levels of TSH, free thyroxin (fT4), TPO Ab and PCA were determined. fT4 was measured by competitive immunoassay on the Roche Elecsys 2010 (electrochemiluminescence immunoassay - ECLIA). TSH was measured by means of a third generation two-site sandwich ECLIA on the Roche Elecsys 2010. The reference range was 11.5–22.7 pmol/L for fT4 and 0.35–5.5 mIU/ml for TSH. TPO Abs were measured using ELISA with commercially available kits (The Binding Site LTD, England); serum TPO Abs levels greater than 150 U/ml were considered as positive.

PCa were measured by ELISA using commercially available kits (ORGENTEC Diagnostica GmbH, Mainz, Germany) according to the manufacturer’s instructions. The results were calculated by reference to the standard curve and expressed as U/ml. To establish the normal upper limit to our laboratory, we have selected the mean + 2 SD (19 U/ml) measured in normal subjects.

Patients with relevant gastric symptoms (dysphagia, unexplained loss of weight, atypical chest pain) underwent gastric endoscopy, followed and confirmed by the histological examination as atrophic gastritis.

The diagnosis of microcytic anemia was based on the simultaneous presence of decreased hemoglobin level (< 120 g/L for women and < 136 g/L for men) and mean corpuscular volume (< 80 fl), which were associated with a low level of serum iron (< 8.8 μmol/L) and high level of total iron binding capacity (> 71.7 μmol/L). All patients with microcytic anemia were assessed for other causes of iron deficiency and obvious causes of blood loss were carefully checked. The diagnosis of macrocytic anemia was based on decreased hemoglobin levels and a mean corpuscular volume greater than 100 fl.

STATISTICS
Statistical analysis was carried out using SPSS, v. 16.0. The results were reported as means ± SEM. Student’s t-test or non-parametric Mann Whitney U test were used to determine whether differences between means were significant. Correlations between the different parameters were calculated by linear regression analysis. Subgroup percentages were compared using the Fisher exact test. Spearman’s rank correlation test was used for the correlation test. p ≤ 0.05 was considered statistically significant.

RESULTS
The relevant clinical and biochemical data for all HT patients, Graves’ disease patients studied and controls are presented in Table 1.
The clinical and biochemical data of subgroups of Hashimoto’s thyroiditis patients are presented in Table 2.

The percentage of PCA positive patients and controls are presented in Fig. 1. PCA positivity was found in 51 patients with autoimmune thyroid diseases, with an overall prevalence of 34 ± 3.9% (in HT patients, 33 ± 4.0% and in GD patients, 43 ± 13.2%, p = 0.6). No significant differences in PCA were found between the groups of HT patients (group I, 32 ± 7.3%; group II, 36 ± 9.6%; group III, 32 ± 5.5%, respectively, p = 0.9). The frequency of PCA in both genders was similar and there were no difference depending on age; patients were divided in two groups –group I < 49-yrs-olds (n = 72) and group II > 49-yrs-old (n = 65) (26 ± 5 U/ml vs 34 ± 6 U/ml; NS).

Fig. 2 shows the mean (± SEM) values of PCA in the study patients and controls. We did not find significant correlation between elevated TPO Abs and PCA in HT patients.

In 10 (7.3 ± 2.2%) of HT patients in different stages of disease we found clinically relevant gastric and/or haematologic symptoms (anemia was present in 8 (80 ± 12.6%) of 10 patients (seven patients with microcytic anemia and one with macrocytic anemia). AG was found with gastroscopy in 4 (all of them had symptoms). In 70% of symptomatic patients PCA were positive (OR = 5.5; 95% CI: 1.2–28.4; p = 0.009).

Table 1. Clinical features of controls, Hashimoto’s thyroiditis (HT) and Graves’ disease (GD) patients included in the study.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (1)</th>
<th>HT (2)</th>
<th>GD (3)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>23</td>
<td>137</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n (% ± Sp)</td>
<td>-</td>
<td>8 (6 ± 2.0)</td>
<td>1 (7 ± 6.8)</td>
<td></td>
</tr>
<tr>
<td>Female n (% ± Sp)</td>
<td>23 (100%)</td>
<td>129 (94 ± 2.0)</td>
<td>13 (93 ± 6.8)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>49.0 ± 2.4</td>
<td>49.0 ± 1.2</td>
<td>51.0 ± 4.2</td>
<td></td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>1.3 ± 0.8</td>
<td>14.4 ± 4.3</td>
<td>0.05 ± 0.01</td>
<td>0.35–5.5</td>
</tr>
<tr>
<td>fT4 (pmol/L)</td>
<td>15.8 ± 0.6</td>
<td>12.8 ± 2.8</td>
<td>32.2 ± 4.1</td>
<td>12–22</td>
</tr>
<tr>
<td>TPO Abs positive patients (%)</td>
<td>-</td>
<td>73% ± 3/8</td>
<td>72% ± 12.0</td>
<td>*2 vs 1 *3 vs 1</td>
</tr>
</tbody>
</table>

Statistical significance: * p < 0.05. Values are given as mean ± SEM.

Table 2. Baseline characteristics in Hashimoto’s thyroiditis patients – euthyroid group (Group I), hypothyroid group (Group II) and a levothyroxine-treated group (Group III) (mean ± SEM)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group I (1)</th>
<th>Group II (2)</th>
<th>Group III (3)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>41</td>
<td>25</td>
<td>71</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male n (% ± Sp)</td>
<td>-</td>
<td>1 (4% ± 3.9)</td>
<td>7 (11% ± 3.7)</td>
<td></td>
</tr>
<tr>
<td>Female n (% ± Sp)</td>
<td>41 (100%)</td>
<td>24 (96% ± 3.9)</td>
<td>64 (89% ± 3.7)</td>
<td></td>
</tr>
<tr>
<td>Age (mean ± SEM)</td>
<td>49.6 ± 1.8</td>
<td>46.6 ± 2.9</td>
<td>45.9 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>TSH (mIU/L)</td>
<td>2.1 ± 0.9</td>
<td>18.9 ± 4.7</td>
<td>3.1 ± 1.8</td>
<td>0.35–5.5</td>
</tr>
<tr>
<td>fT4 (pmol/L)</td>
<td>15.1 ± 2.4</td>
<td>9.5 ± 2.6</td>
<td>17.3 ± 3.4</td>
<td>12–22</td>
</tr>
<tr>
<td>TPO Abs positive patients (%)</td>
<td>61% ± 7.6</td>
<td>94% ± 4.7</td>
<td>75% ± 5.1</td>
<td>*1 vs 2 *1 vs 2</td>
</tr>
</tbody>
</table>

Statistical significance: * p < 0.05.
Clinical Significance of Autoantibodies to Parietal Cells in Patients with Autoimmune Thyroid Diseases

Figure 1. Percentage of PCA (autoantibodies to the parietal cells) positive patients and controls: HT (Hashimoto’s thyroiditis); GD (Grave’s disease); * p < 0.05.

Figure 2. Mean (± SEM) values of PCA (autoantibodies to the parietal cells) in study patients and controls: HT (Hashimoto’s thyroiditis), GD (Grave’s disease); * p < 0.05.
PCA positive HT patients required higher LT4 doses than PCA negative (1.46 μg/kg vs 1.24 μg/kg, p = 0.04). In a whole group of treated HT patients (n = 71) a significant positive correlation was found between the daily LT4 requirement and the levels of serum PCA (r = 0.28; p = 0.017). We found no differences of the required doses depending on age and sex of HT patients.

DISCUSSION

This study showed a PCA prevalence of 33.8% in patients with autoimmune thyroid diseases - in HT patients - 33% and in GD patients - 43%, which is similar to the results found in previous studies and higher than that in general population. In this study the prevalence of PCA in the control group was 9% (2/23 women), but positive results were in equivocal concentrations.

Advancing age is a risk factor that has been associated with PCA positivity. In general population, PCA positivity increases from 2.5% in the third decade to 12% in the eighth decade. In HT patients there were no difference depending on age, PCA were present in same concentration in the group of patients younger than 49 yrs and in the group of patients older than 49 yrs.

To our knowledge, there have been only a few studies addressing the issue of PCA in HT patients in different stage of functional activity of the disease. In our study we found similar frequency of PCA positivity in HT patients regardless of their functional activity. Segni et al. reported that the difference between PCA positive and PCA negative HT juvenile patients needing a treatment for hypothyroidism did not reach statistical significance. We may conclude that PCA, as a marker of gastric autoimmunity, may be present in early euthyroid stage of HT. Early detection of PCA and autoimmune gastritis is important in preventing iron deficiency anemia, which may influence work capacity and cardiopulmonary status, and pernicious anemia, which can cause neurological complications and (pre)malignant gastric lesions. For patients with ATD, it seems appropriate to test PCA status at the diagnosis of disease or in any other time if there are clinical indications (gastric or haematologic symptoms, treated HT patients requiring higher levothyroxine dose to achieve a serum TSH level within the normal range) because the test may later become positive. We confirm results of Checchi et al. that the LT4 requirement was significantly higher in PCA-positive than in PCA-negative patients. The doses required do not depend on the sex and age of HT patients. The likely mechanism reducing LT4 absorption in PCA-positive patients may be related to the impaired chlorhydric acid secretion as a result of the chronic damage of gastric mucosa and particularly of the reduced number and function of the oxyntic glands. Thus, reduction of gastric acidity may be responsible for reduced absorption of LT4.

The presence of relationship between thyroid antibodies and gastric disorders remains contradictory. In a recent study Garcia et al. showed that elevated levels of antithyroid antibodies increases the risk of PCA positivity. The lack of correlation between TPO Abs levels and levels of APA in our work may be due to different ways of appearance of these antibodies. Tozzi et al. have found that PCA levels rise progressively over time, reach a peak level and then fall, according to the progressive destruction of gastric mucosa and to the disappearance of the target autoantigen (proton pump), while TPO Abs appear involved in the tissue destructive processes associated with the hypothyroidism observed in Hashimoto's thyroiditis. The appearance of TPO Abs usually precedes the development of thyroid dysfunction. In a previous study we found that the TPO Abs concentrations in euthyroid patients were significantly lower in comparison with both hypothyroid patients and levothyroxine-treated patients. After treatment with thyroid hormones the serum levels of TPO Abs declined. Ito et al. also found significantly higher titers of TPO Abs in HT patients with overt hypothyroidism than in those with euthyroidism.

In this study in 7.3% of HT patients we found, in different functional stages of disease, clinically relevant gastric and/or haematologic symptoms (anemia was present in 80% of them (70% - with microcytic anemia and 10% - with macrocytic anemia), AG was found with gastroscopy in 4 patients (all of them had symptoms). In 70% of symptomatic patients PCA were positive. Atrophic body gastritis is an asymptomatic disease and discrepancies in the prevalence depend on methods used for diagnosis. Centanni et al. found hypergastrinemia to be a marker of AG in 35% of patients with ATD and the diagnosis was histologically confirmed. Anemia was observed in 82% of patients with ATD and atrophic body gastritis and was predominantly microcytic. If we consider these results in conjunction with our results we can conclude that in HT patients even the presence of undiagnosed unexplained microcytic anemia should be considered as highly suggestive of the presence...
of undiagnosed AG. This assumption is in keeping with the finding that hypochlorhydria may lead to iron deficiency and anemia. In this respect, all patients with ATD should be routinely monitored at follow-up for the presence of anemia which may be the only clinical sign of undiagnosed AG.

CONCLUSIONS

In conclusion, PCA may be present in different stages in HT patients and this does not depend on the severity of disease. PCA concentrations may predict symptoms of atrophic gastritis in patients with Hashimoto’s thyroiditis. PCA positive HT patients require higher replacement doses of LT4. The presence of anemia, particularly microcytic anemia, was suggestive of undiagnosed atrophic gastritis.

REFERENCES

БЕИНН: Клиническое значение наличия аутоантител к париетальным клеткам (АПА) у пациентов с аутоиммунными тиреоидными заболеваниями (АТИЗ) противоречиво.

Цель: Установить частоту АПА у пациентов с АТИЗ; выяснить роль возраста и пола для наличия АПА; анализировать ассоциацию АПА с наличием гематологических или желудочных проявлений, как и с дозой Levothyroxine, необходимой для достижения еутиреоидного состояния пациентов с тиреоидитом Хашимото – ТХ.

ПАЦИЕНТЫ И МЕТОДЫ: АПА исследованы с помощью ELISA у 137 пациентов с ТХ, разделенных в зависимости от своего функционального состояния на 3 подгруппы: группа 1 включает пациентов с нормальной функцией щитовидной железы; группа 2 – пациентов с гипотиреоидизмом; группа 3 включает пациентов с гипотиреоидизмом, леченных Levothyroxine (LT4) в дозе, поддерживающей TSH и fT4 в нормальных значениях. Исследовано также наличие АПА у 14 пациентов с активной Базедовой болезнью и 23 здоровых лиц в качестве контрольной группы.

РЕЗУЛЬТАТЫ: Наличие АПА установлено у 51 пациента с АТИЗ – 33.8%. Статистически значимые различия между наличием АПА в различных подгруппах пациентов с ТХ не установлены. Частота АПА близка для обоих полов. Зависимость от возраста не наблюдалась. В 7.3% случаев пациентов с ТХ в различных стадиях активности заболевания установлены клинически значимые желудочные или гематологические симптомы; в 70% АПА позитивны. (OR = 5.5; 95% CI: 1.2 – 28.4; p = 0.009). Леченные пациенты с ТХ, позитивные на АПА нуждались в более высоких дозах LT4 по сравнению с АПА негативными (1.46 μg/kg к 1.24 μg/kg, p = 0.04).

ЗАКЛЮЧЕНИЕ: АПА могут наблюдаться во время различных стадий активности ТХ и это не зависит от тяжести заболевания. Наличие АПА может предшествовать симптомы атрофического гастрита у пациентов с ТХ, пациенты с ТХ, позитивные на АПА, нуждаются в более высоких дозах LT4. Наличие анемии, особенно микроцитарной, может быть проявлением недиагностированного атрофического гастрита.