The Amsterdam autoimmune thyroid disease cohort
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Stress is not associated with thyroid peroxidase autoantibodies in euthyroid women

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Chapter 5
Stress is not associated with thyroid peroxidase autoantibodies in euthyroid women

Abstract

Objective: Multiple genes and environmental factors play a role in the etiology of Autoimmune Thyroid Disease (AITD). In Graves' hyperthyroidism, stress is such an environmental factor, but whether it plays a role in Hashimoto’s hypothyroidism is unknown. We used validated questionnaires to evaluate an association between TPO antibodies, an early marker for AITD, and self-reported stress.

Subjects and methods: Recently Experienced Stressful Life Events, Daily Hassles and mood (tendency to report positive and negative affects) were assessed in 759 euthyroid subjects.

Results: TPO antibodies were found in 183/759 (24%) of subjects. The TPO-Ab positive subjects were older (39.7±12 vs. 34.2±12 years; p<0.001) than the TPO-Ab negative subjects, but the number of daily hassles (24±14 vs. 25±14; p=0.24), the number of stressful life events (10±6 vs. 11±6; p=0.09) and the scores on the affect scales (22.1±7.4 vs. 22.2±7.3; p=0.89 for negative affect and 38.2±5.1 vs. 38.3±5.3; p=0.91 for positive affect) were similar in TPO-Ab positive and TPO-Ab negative subjects.

Conclusions: We found no association between recently experienced stressful life events, daily hassles or mood and the presence of TPO antibodies in euthyroid subjects.
Chapter 5

Introduction

The etiology of autoimmune thyroid disease (AITD), encompassing Graves' hyperthyroidism and Hashimoto’s hypothyroidism is multifactorial. It has been estimated that 79% of the liability to develop Graves’ disease can be attributed to genes (Brix et al., 2001); therefore other risk factors must play a role (Weetman & McGregor, 1994). Among environmental factors, stress has been implicated as a risk factor for the development of Graves’ disease (Winsa et al., 1991; Dayan, 2001). Stress has a profound effect on the immune system (reviewed by Elenkov and Chrousos, 2002) and we have reported previously that self-reported life stress influences the number of circulating T lymphocytes (Brosschot et al., 1994). We therefore wondered whether stress might also be associated with Hashimoto’s thyroiditis. The earliest event in Hashimoto’s thyroiditis is the occurrence of TPO antibodies and we therefore compared stress/levels between 183 women with TPO antibodies and 576 without.

Subjects and methods

The study cohort consisted of the 759 female subjects (age 18-65 years at study entrance) from the Amsterdam AITD cohort who were biochemically euthyroid at study entrance (Strieder et al., 2003). All subjects were in self-proclaimed good health, without a history of thyroid disease. They were asked to complete three questionnaires.

The Dutch questionnaire on Recently Experienced Stressful Life Events (Brosschot et al., 1994; van de Willige et al., 1985) counts the total number of major life events experienced in the past 12 months (checklist of 60 possible events). The respondent scores separately the amount of pleasantness and unpleasantness associated with each experienced life event, rated on a scale from zero (meaning no (un) pleasantness at all) to four (a huge amount of (un) pleasantness). From this scale, the total amount of pleasantness and unpleasantness is calculated (maximal score 240 for each). When the amount of pleasantness exceeds the amount of unpleasantness the event is categorized as being pleasant and vice versa, yielding the total number of (un) pleasant events (maximum 60).

The Dutch Everyday Problem Checklist, a validated version of the Daily Hassles Scale (Kanner et al., 1981; Vingerhoets et al., 1989, 1996), consists of 114 items concerning daily hassles experienced in the last 2 months. It also measures the intensity of each hassle on a scale from zero to three, yielding the number of hassles experienced and the total intensity of these hassles (maximum 342).
The Positive and Negative Affect Schedule (PANAS; Watson et al., 1988) measures the current mood, in terms of positive and negative affect. It consists of 22 mood states (11 positive, 11 negative) and the respondent is asked to report whether she is affected by each of these states on a scale from 1 (not at all) to 5 (a lot). This yields the tendency to report positive and negative affect states both on a scale from 11 to 55.

Information concerning the use of psychotropic medication was obtained via a medical questionnaire. In addition, educational level (on a 4-point scale) and employment (having a paid job) status was also obtained. Blood samples were drawn and coded to ensure confidentiality and serum was stored at -20°C until assay. The concentration of antibodies against TPO and thyroglobulin (Tg) was measured using a chemiluminescence immunoassay (LUMI-test, Brahms, Berlin, Germany), and a value of $\geq 100$ kU/L was defined as positive. Concentration of free thyroxine was assayed by time-resolved fluoroimmunoassay (fT4, Delfia, Turku, Finland) and concentration of thyrotropin by immunoradiometric assay (TSH, Delfia). For this study, euthyroidism was defined as a TSH value between 0.40 and 5.70 mU/L (Strieder et al., 2003).

The study was approved by the local Medical Ethics committee and all subjects gave their written informed consent.

Statistical analysis was done using the SPSS 10.0 package. Mean values were compared between subjects with and those without TPO antibodies using two-sided, unpaired Student’s t test, median values using Mann-Whitney U test; rates were compared using $\chi^2$ (or if appropriate Fishers’ exact) tests. Because age is a known risk factor for a positive TPO antibody status (Strieder et al., 2003) comparison between groups was adjusted for the age difference using linear regression analysis.

**Results**

Subjects with TPO antibodies as compared to those without were older and had higher TSH levels, whereas free T4 values were similar in both groups as reported previously (Strieder et al., 2003). There were no differences in educational level, employment status, or the use of psychotropic drugs (Table 1). As for the stress variables (Table 2), we observed no differences in the various stress measurements between subjects with and without TPO antibodies. Women with TPO antibodies reported a lower number of pleasant events and less total pleasantness than females without TPO antibodies, but this was fully accounted for by the age difference. We also did not find any relation between stress and the level or presence.
Table 1. Characteristics of 759 female euthyroid relatives of AITD patients

<table>
<thead>
<tr>
<th></th>
<th>TPO-Ab pos</th>
<th>TPO-Ab neg</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>183</td>
<td>576</td>
<td></td>
</tr>
<tr>
<td>Age (mean+SD) in years</td>
<td>39.7 ± 12</td>
<td>34.2 ± 12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TSH (median+range) in mU/l</td>
<td>2.3 (0.51-5.70)</td>
<td>1.6 (0.42-5.70)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FreeT4 (median+range) in pmol/l</td>
<td>12.5 (7.6-42.7)</td>
<td>12.9 (7.2-21.5)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Highest obtained level of education
- elementary school (low level of education) 6 (3%) 20 (3%) 0.90
- high school (medium level of education) 55 (30%) 139 (24%) 0.11
- high school (high level of education) 65 (36%) 246 (43%) 0.09
- university (very high level of education) 56 (31%) 170 (30%) 0.78

Employed 123 (67%) 404 (70%) 0.45
On psychotropic drugs 4 (2.2%) 12 (2.1%) 0.9
On psychotropic drugs in the previous year 9 (5%) 20 (3.5%) 0.4

p-Value for age was obtained by Students’ t test, p-values for TSH and freeT4 were obtained by Mann-Whitney U test, p-values for the other variables were obtained by χ² test. TPO-Ab positive: TPOAb ≥ 100kU/l.

of Tg antibodies (data not shown). There were no other differences among both groups in the three questionnaires.

Discussion

The rationale for our study was the observation that patients with Graves’ disease experienced more negative life events than controls in several case-control studies (Kung, 1995; Matos-Santos et al., 2001; Winsa et al., 1991), and that stress has a profound effect on the immune system (Brosschot et al., 1994; Elenkov and Chrousos, 2002; Sternberg, 2001). We hypothesized that stress may also be associated with the presence of TPO antibodies, an early marker for Hashimoto’s thyroiditis (Prummel and Wiersinga, 2002). We used two different and well-validated questionnaires and found no relation between and antibody status in 759 euthyroid females.

One source of bias could have been the Affect State because mood may alter the recall of stressful events. We therefore also assessed the Affect State using the PANAS questionnaire and found no differences in mood between the two groups, also not after correction for age. Age, however, was a definite confounder. The TPO positive women were 5 years older than
**Table 2.** Experienced stress in TPO-Ab positive and TPO-Ab negative euthyroid subjects (values as mean with SD between parentheses)

<table>
<thead>
<tr>
<th></th>
<th>TPO-Ab pos</th>
<th>TPO-Ab neg</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>N</strong></td>
<td>183</td>
<td>576</td>
<td></td>
</tr>
<tr>
<td><strong>Recent Life Events</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- total number of life events</td>
<td>10.3 (6.1)</td>
<td>11.2</td>
<td>0.09</td>
</tr>
<tr>
<td>- number of unpleasant events</td>
<td>4.6 (3.5)</td>
<td>4.7 (3.4)</td>
<td>0.68</td>
</tr>
<tr>
<td>- number of pleasant events</td>
<td>4.5 (3.5)</td>
<td>5.2 (3.5)</td>
<td>0.02</td>
</tr>
<tr>
<td>- amount of total unpleasantness</td>
<td>15.1 (11.0)</td>
<td>16.2</td>
<td>0.13</td>
</tr>
<tr>
<td>- amount of total pleasantness</td>
<td>15.9 (11.4)</td>
<td>16.2</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Daily Hassles</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- total number of daily hassles</td>
<td>23.8 (13.6)</td>
<td>25.0</td>
<td>0.24</td>
</tr>
<tr>
<td>- intensity per hassle</td>
<td>1.3 (0.4)</td>
<td>1.3</td>
<td>0.52</td>
</tr>
<tr>
<td>- total intensity of all hassles</td>
<td>32.3 (22.9)</td>
<td>34.2</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Positive and Negative Affect Schedule Scale</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- tendency to report negative feelings</td>
<td>22.1 (7.4)</td>
<td>22.1</td>
<td>0.89</td>
</tr>
<tr>
<td>- tendency to report positive feelings</td>
<td>38.2 (5.1)</td>
<td>38.3</td>
<td>0.91</td>
</tr>
</tbody>
</table>

* Corrected for age by linear regression analysis.
** p Value after correction for age.

the TPO negative subjects and van de Willige et al. (1985) have shown that differences in age are associated with the experienced amount and type of the studied indicators of self-reported stress (the older one becomes the less pleasant and less unpleasant events one experiences). This is in agreement with our findings that the older TPO positive women reported less life events and daily hassles than the younger TPO negative women (Table 2).

Another possible confounding factor is the unknown duration of the presence of TPO antibodies, whereas we measured stress experienced in the preceding 2-12 months. Although even if TPO antibodies were developed a longer time ago, one may have expected a relation between stress and the concentration of TPO antibodies which we also could not establish. Nevertheless, to completely rule out a role of stress a prospective study in TPO antibody negative women developing TPO antibodies is needed.
Acknowledgment

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References