Prevalence of Serum Anti-Myeloperoxidase Antineutrophil Cytoplasmic Antibodies (MPO-ANCA) in Patients with Graves’ Disease Treated with Propylthiouracil and Thiamazole

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Abstract. Patients with Graves’ disease (n = 61) treated with propylthiouracil (PTU) or thiamazole (MMI) were studied retrospectively to investigate differences in the prevalence of anti-myeloperoxidase anti-neutrophil cytoplasmic antibodies (MPO-ANCA) in relation to treatment with anti-thyroid drugs. The patients were divided into two groups: PTU-treated group (n = 32) and MMI-treated group (n = 29). There were no significant differences between the two groups in terms of age, gender distribution, or duration of treatment. In the PTU group, 8/32 (25%) patients were positive for MPO-ANCA, whereas in the MMI group, 1/29 (3.4%) patients were positive. There were no significant differences in age, duration, or dosage between the MPO-ANCA positive and negative patients. Most of the MPO-ANCA positive patients were asymptomatic, except for two patients in whom rheumatic arthritis or membranous glomerulonephritis developed. None of the MPO-ANCA positive patients were diagnosed as having classical ANCA-associated vasculitis. Thus, there is a high frequency of MPO-ANCA in patients with Graves’ disease treated with PTU, compared with patients treated with MMI, although classical ANCA-associated vasculitis develops in only a few MPO-ANCA positive patients.

Key words: Propylthiouracil, Anti-myeloperoxidase antineutrophil cytoplasmic antibody, ANCA-associated vasculitis (Endocrine Journal 49: 329-334, 2002)

ANTI-NEUTROPHIL cytoplasmic antibodies (ANCA) are associated with small vessel vasculitis, including Wegener’s granulomatosis, microscopic polyangiitis, Churg-Strauss syndrome and primary pauci-immune necrotizing and crescentic glomerulonephritis [1]. Two specific types of ANCA have been demonstrated by using indirect immunofluorescence of alcohol fixed neutrophils: one staining with a centralized cytoplasmic pattern (C-ANCA), and one with a perinuclear pattern (P-ANCA). C-ANCA is directed at proteinase 3 (PR-3), and is highly specific for Wegener’s granulomatosis [2]. P-ANCA is often directed at human neutrophil myeloperoxidase (MPO), and is frequently associated with pulmonary-renal syndrome and necrotizing and crescentic glomerulonephritis [3].

The triggers for the development of ANCA-associated vasculitis are unknown. However, many cases of drug-induced ANCA-associated vasculitis have been reported. Most of these have been associated with MPO-ANCA. Hydralazine and propylthiouracil (PTU) have been most often implicated in the induction of ANCA-associated vasculitis [4]. There have also been many cases of PTU-induced ANCA-associated vasculitis [5-21]. Most of these
were in Japanese people [5-11]. For anti-thyroid drugs (ATD), there are at least two reports of thiamazole (MMI)-induced ANCA-associated vasculitis [22, 23]. On the other hand, the prevalence of MPO-ANCA positive subjects among patients treated with ATD has been reported only rarely [24, 25].

In this study, the prevalence of serum MPO-ANCA was examined in two groups of patients with Graves’ disease, treated with ATD, PTU or MMI, and the clinical findings of patients with MPO-ANCA are reported. We found a higher prevalence of positive MPO-ANCA in the group of patients treated with PTU.

**Subjects and Methods**

We studied 61 Japanese patients with Graves’ disease treated with ATD (five men and 56 women, mean age 41.2 ± 12.0 years). The patients were divided into two groups according to the drugs administered: a PTU group and a MMI group.

Serum MPO-ANCA concentrations were measured using an enzyme-linked immunosorbent assay (ELISA: Nissho Co., Osaka, Japan). The cut-off value recommended by this commercial kit was 20 EU. Serum ANCA was measured using an immunofluorescence assay (IFA: Inova Diagnostics Inc., San Diego, CA, USA). Serum levels of antithyroglobulin antibodies (TgAb) and anti-thyroid peroxidase antibodies (TPOAb) were measured by radioimmunoassay (RIA: RSR Ltd., Cardiff, UK). TSH binding inhibitory immunoglobulin (TBI) levels were measured by radio receptor assay (DiaSorin Inc., Stillwater, MN, USA). Serum free triiodothyronine (FT3), free thyroxine (FT4) and thyrotropin (TSH) levels were measured by enzyme immunoassay (EIA: Boehringer Mannheim, Mannheim, Germany).

For statistical analysis, Mann-Whitney’s non-parametric U test was performed for comparisons between two groups. Discrete data were analyzed by chi-square test. Regression analysis was performed to determine any association between parameters. A p value of less than 0.05 was considered significant in each test.

**Results**

In the PTU group, eight of the 32 patients (25.0%) were positive for MPO-ANCA in serum, whereas in the MMI group, only one of the 29 patients (3.4%) was positive. The prevalence of MPO-ANCA was significantly higher in the PTU group (p = 0.02).

Table 1 summarizes the background of the patients. There were no significant differences in age, gender distribution or duration of treatment between the two groups. Table 2 shows the parameters associated with Graves’ disease in the PTU group. There were no significant differences in TgAb, TPOAb, TBI, FT3, or FT4 levels between MPO-ANCA positive and negative patients. Serum TSH levels were significantly higher in the MPO-ANCA positive patients (p<0.05), although mean serum TSH levels were normal in both subgroups. In eight patients with positive MPO-ANCA in the PTU group, the level of MPO-ANCA was not significantly correlated with that of TPOAb (p>0.05). When data for all 61 patients were pooled, there were no significant differences in age, duration of treatment, or TgAb, TPOAb, FT3, or FT4 levels between MPO-ANCA positive and negative patients, except for lower TBI levels and higher TSH levels in the MPO-ANCA positive patients (p<0.05).

The clinical findings for the MPO-ANCA positive patients are summarized in Table 3. The concentrations of MPO-ANCA ranged from 22 to 48 EU. Eight of the nine patients with positive serum MPO-

| Table 1. The background of 61 patients with Graves' disease treated with anti-thyroid drugs. |
|---------------------------------------------|---------------------------------------------|-------------|
| PTU group (n = 32) | MMI group (n = 29) | P value |
| Gender distribution | 3 men, 29 women | 2 men, 27 women | 0.72 |
| Age (year) | 41 ± 13 | 41 ± 11 | 0.96 |
| Duration of treatment (month) | 60.3 ± 50.0 | 59.3 ± 65.6 | 0.95 |
| Dosage of ATD (mg/day) | 156 ± 103 | 13 ± 9 |  |

The data are shown as mean ± SD for the propylthiouracil-treated group and the thiamazole-treated group. PTU: propylthiouracil. MMI: thiamazole. ATD: anti-thyroid drugs.
Table 2. Parameters related to Graves' disease in MPO-ANCA positive and negative patients treated with propylthiouracil.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Positive patients (n = 8)</th>
<th>Negative patients (n = 24)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>42 ± 11</td>
<td>41 ± 13</td>
<td>0.85</td>
</tr>
<tr>
<td>Gender distribution</td>
<td>8 women</td>
<td>3 men, 21 women</td>
<td>0.29</td>
</tr>
<tr>
<td>Duration of treatment (month)</td>
<td>53.9 ± 34.0</td>
<td>62.4 ± 54.8</td>
<td>0.68</td>
</tr>
<tr>
<td>Dosage of PTU (mg/day)</td>
<td>106 ± 86</td>
<td>172 ± 104</td>
<td>0.07</td>
</tr>
<tr>
<td>FT3 (pg/ml)</td>
<td>4.48 ± 0.35</td>
<td>5.10 ± 1.22</td>
<td>0.17</td>
</tr>
<tr>
<td>FT4 (ng/dl)</td>
<td>1.27 ± 0.25</td>
<td>1.37 ± 0.40</td>
<td>0.45</td>
</tr>
<tr>
<td>TSH (μU/ml)</td>
<td>2.10 ± 1.55</td>
<td>0.84 ± 1.29</td>
<td>0.01</td>
</tr>
<tr>
<td>TgAb (U/ml)</td>
<td>12.0 ± 26.8</td>
<td>13.4 ± 26.6</td>
<td>0.93</td>
</tr>
<tr>
<td>TPOAb (U/ml)</td>
<td>6.0 ± 6.2</td>
<td>8.0 ± 6.9</td>
<td>0.50</td>
</tr>
<tr>
<td>TBI (%</td>
<td>1.0 ± 1.8</td>
<td>15.9 ± 24.8</td>
<td>0.05</td>
</tr>
</tbody>
</table>

The data are presented as mean ± SD. PTU: propylthiouracil. MPO: myeloperoxidase. FT3: free triiodothyronine (normal 3.8-6.1 pg/ml). FT4: free thyroxine (normal 0.9-1.9 ng/dl). TSH: thyrotropin (normal 0.5-5.0 μU/ml). TgAb: thyroglobulin antibodies (normal < 0.3 U/ml). TPOAb: anti-thyroid peroxidase antibodies (normal < 0.3 U/ml). TBI: TSH binding inhibitory immunoglobulin (normal < 10%).

Table 3. Clinical findings for the MPO-ANCA positive patients

<table>
<thead>
<tr>
<th>Patients No.</th>
<th>Age (year)</th>
<th>Gender</th>
<th>Drug</th>
<th>Dosage (mg/day)</th>
<th>Duration (month)</th>
<th>MPO-ANCA (EU)</th>
<th>ANCA</th>
<th>Urine protein</th>
<th>Serum creatinine (mg/dl)</th>
<th>Complication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32</td>
<td>F</td>
<td>PTU</td>
<td>100</td>
<td>84</td>
<td>30</td>
<td>3+</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>54</td>
<td>F</td>
<td>PTU</td>
<td>50</td>
<td>16</td>
<td>22</td>
<td>2+</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>F</td>
<td>PTU</td>
<td>50</td>
<td>60</td>
<td>40</td>
<td>1+</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>4</td>
<td>33</td>
<td>F</td>
<td>PTU</td>
<td>300</td>
<td>36</td>
<td>31</td>
<td>1+</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>5</td>
<td>32</td>
<td>F</td>
<td>PTU</td>
<td>50</td>
<td>12</td>
<td>22</td>
<td>1+</td>
<td>-</td>
<td>-</td>
<td>0.6</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>F</td>
<td>PTU</td>
<td>100</td>
<td>103</td>
<td>31</td>
<td>1+</td>
<td>-</td>
<td>-</td>
<td>0.5</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>F</td>
<td>PTU</td>
<td>50</td>
<td>84</td>
<td>24</td>
<td>-</td>
<td>N.D.</td>
<td>N.D.</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>46</td>
<td>F</td>
<td>MMI</td>
<td>5</td>
<td>9</td>
<td>41</td>
<td>3+</td>
<td>3+</td>
<td>0.7</td>
<td>0.6</td>
</tr>
</tbody>
</table>


ANCA concentrations measured by ELISA also had positive serum P-ANCA measured by IFA. In the two patients with positive MPO-ANCA, one (Patient #3) had rheumatoid arthritis (RA) with an ACR type classification [26]; another (Patient #9) had a nephrotic syndrome, diagnosed as membranous glomerulonephritis (MGN) with microscopic findings from a renal biopsy. This patient, treated with MMI, had been administered PTU for six years previously. The other seven patients had no symptoms of fever, arthralgia, skin eruptions or numbness. In seven of nine MPO-ANCA positive patients, MPO-ANCA was measured 12 months later (Table 4). In six patients, except for Patient #5, serum MPO-ANCA levels were still positive. In Patient #5, PTU had been substituted for MMI after six months and the titer of MPO-ANCA reduced to 12 EU 12 months later. There were no patients in whom new symptoms or complications developed.

Discussion

We found that 25% of patients with Graves' disease treated with PTU showed positive circulating levels of MPO-ANCA, although only 3.4% of age-matched patients treated with MMI had MPO-ANCA. It is clear that the prevalence of MPO-
ANCA in patients treated with PTU is high. In addition, the patient with positive MPO-ANCA treated with MMI had been taking PTU for six years before she began to take MMI. The finding of positive MPO-ANCA in this patient could be related to PTU administration. We suggest that chronic PTU treatment may be strongly associated with the development of MPO-ANCA. Sera et al. reported that MPO-ANCA was detected in 21 of 56 patients (37.5%) with Graves' disease treated with PTU [24]. Sato et al. reported that MPO-ANCA was positive in 16 of 25 patients (64.0%) with childhood onset Graves' disease treated with PTU [25]. It is clear from these reports that the prevalance of MPO-ANCA in patients treated with PTU is higher than in patients treated with MMI.

The pathogenesis of developing MPO-ANCA is not clearly understood. It has been speculated that reactive metabolites of PTU may bind and modify the enzyme to induce antibodies to MPO [27].

In our study, there were no significant differences in age, dosage of PTU, duration of treatment, or measured levels of TgAb and TPOAb between MPO-ANCA-positive and negative patients in the PTU group. The mean serum TSH levels were higher in MPO-ANCA positive patients than in MPO-ANCA-negative patients, although TSH levels were within the normal range in both subgroups. In MPO-ANCA negative patients, seven of 24 had low serum TSH levels, and four had high serum FT3 or FT4 levels. None of the eight MPO-ANCA positive patients had low serum TSH levels (data not shown). In MPO-ANCA negative patients, the control of thyroid function was worse than in MPO-ANCA positive patients. However, it is uncertain whether this was related to the development of MPO-ANCA.

Human MPO and human TPO share global similarities in their nucleotide (46%) and amino acid sequences (44%) [28]. Therefore, it is conceivable that MPO-autoantibodies may cross-react with TPO. There have been reports that several patients suffering thyroid disorders with positive TPOAb titers were also positive for MPO-ANCA [29, 30]. However, in our study, the levels of TPOAb did not differ between MPO-ANCA positive and negative patients. Moreover, the levels of MPO-ANCA were not correlated with those of TPOAb in MPO-ANCA positive patients treated with PTU.

In this study, none of the nine patients with positive MPO-ANCA was diagnosed as having apparent ANCA-associated vasculitis. Two patients developed RA or MGN. However, the relationship between MPO-ANCA and these complications is uncertain. There is a report of MPO-ANCA in patients with RA, but MPO-ANCA is probably not involved in this [31]. The other seven MPO-ANCA positive patients in our study had no symptoms. It is thought that ANCA-associated vasculitis develops in only a few of MPO-ANCA positive patients. Sato et al. also reported that none of the patients with possible MPO-ANCA had clinical manifestations and laboratory findings of vasculitis or nephritis [25]. On the other hand, Sera et al. reported that nine of 21 patients with positive MPO-ANCA complained of myalgia, polyarthralgia, skin purpura, and recurrent common cold-like symptoms associated with vasculi-
tis after PTU therapy. However, none of these patients showed abnormalities in their urine [24].

There are three explanations of the observation that only some patients with positive MPO-ANCA develop ANCA-associated vasculitis. First, most of the patients with PTU-induced ANCA-associated vasculitis also had high titers of MPO-ANCA. Morita et al. reviewed cases of PTU-induced MPO-ANCA-associated vasculitis, and reported that the mean titer of MPO-ANCA was 250 EU and that titers of MPO-ANCA were above 100 EU in two-thirds of the patients [11]. On the other hand, the MPO-ANCA positive patients in the present study had lower levels of MPO-ANCA (all patients had titers lower than 50 EU). Second, asymptomatic patients with positive MPO-ANCA in this study may have only recently developed these antibodies, and apparent ANCA-associated vasculitis may possibly develop in the future; however, none of the MPO-ANCA positive patients had developed MPO-ANCA-associated vasculitis for the 12 months of this study. Third, there may be a difference between drug-induced and vasculitis-associated patients in the epitope on the MPO molecule recognized by MPO-ANCA. The epitope recognized by MPO-ANCA is associated with the clinical features of the ANCA-associated disease [32]. There are no reports of epitopes in asymptomatic subjects with positive MPO-ANCA. The different epitopes on MPO recognized by each class of MPO-ANCA should be resolved in the future.

This was a retrospective study. A prospective study is required to evaluate the timing of development of MPO-ANCA after the administration of PTU. Moreover, long time observation of asymptomatic MPO-ANCA positive patients is necessary for a rigorous study.

In summary, a quarter of our patients with chronic administration of PTU were positive for MPO-ANCA, although only a few of them develop ANCA-associated vasculitis.

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References


