Evidence for acute release of thyroid peroxidase during subtotal thyroidectomy

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Abstract. An immediate reduction of thyroglobulin autoantibodies during subtotal thyroidectomy of thyroglobulin antibody positive patients has previously been shown to indicate an acute release of thyroglobulin into the circulation peroperatively. The aim of the present study was to investigate whether thyroid peroxidase was also released by measuring anti-thyroid peroxidase antibodies by a quantitative and antigen specific method both pre- and postoperatively in patients positive for anti-thyroid peroxidase antibodies. Twelve anti-thyroid peroxidase positive patients (11 females, 1 male) referred for surgery of toxic goitre were studied. Median age was 43 years (range 24-64) and median goitre size 86 g (25-165). All patients had been pretreated with antithyroid drugs and were euthyroid at the time of operation. Anti-thyroid peroxidase was measured before operation, 1-8 h, 10 days, 1-3 months, and 12 months postoperatively by a commercial method (DYNOSTE®). The median anti-thyroid peroxidase level before operation was 1048 kU/l (range 68-10 517 kU/l) and fell during operation to 0.63 (range 0.37-1.28) (p<0.01) of initial concentration without further decrease during the next 1-8 h. The comparative decrease in thyroglobulin antibodies was 0.19 (0-0.88). The anti-thyroid peroxidase level was increasing after 10 days, but did not reach initial level until between 3 and 12 months after surgery. However, in 3 of 10 patients anti-thyroid peroxidase had disappeared after 12 months, all of whom had low levels before operation, whereas anti-thyroid peroxidase was 2-4 times higher than preoperatively in 3 other patients. The present study thus gives evidence for an acute release of thyroid peroxidase into the circulation during thyroid surgery able to decrease anti-thyroid peroxidase activity to the same degree as the Tg-induced decrease in Tg-ab.

It has been shown that during subtotal thyroidectomy, thyroglobulin (Tg) is released immediately into the circulation (1,2) and a concomitant reduction of thyroglobulin autoantibodies (Tg-ab) in Tg-ab positive patients has been taken as an indirect evidence of this phenomenon (1,3). Possibly, also other thyroid antigens are released. Measurement of thyroid stimulating immunoglobulins directed against the TSH receptor has given conflicting results depending on the method of measurement (4). Evaluation of microsomal antibodies (Mi-ab) has only been performed in few patients, and with conflicting results (5,6), most likely owing to the use of different methods for measurement of Mi-ab.

Since the specific antigen of the Mi-ab reaction is now known to be due to thyroid peroxidase (TPO), the aim of the present study was to use a recently introduced quantitative and antigen-specific method for measurement of anti-thyroid peroxidase antibodies (anti-TPO) (6) to get indirect evidence for the behaviour of TPO during and after thyroid surgery.
Patients and Methods

Twenty-eight consecutive patients with thyrotoxicosis (13 with Graves' disease, 15 with toxic multinodular goitre) referred for surgery entered the study initially, after informed consent. Twelve of the patients (9 with Graves' disease, 3 with toxic multinodular goitre) had anti-TPO above 10 kU/l and were included for further study during and after operation. They were 11 females and 1 male, with a median age of 43 years (range 24-64) and a median goitre size of 86 g (25-165), as indicated by removed goitrous tissue. All patients had been pretreated with antithyroid drugs and were euthyroid at the time of operation. Anti-TPO was measured before operation, 1-8 h, 10 days, 1-3 months, and 12 months postoperatively by a commercial method (DYNO-test®; Henning, Berlin). The method is based on a radioimmunoassay principle described by Ruf et al. (7). The intra-assay variation was 3-5% and the total-assay variation 6-19% depending on the level (150-1500 kU/l). Serum concentrations of thyroxine, triiodothyronine, TSH, Tg-ab, Mi-ab and the T3-uptake test were measured as previously described (8,9).

Antinuclear antibodies (ANA) and IgM rheumatoid factor (IgM-RF) were measured as described previously (9,10). Serum was stored at −20°C until analysis, and serum from one patient was run in the same assay.

Wilcoxon's test for paired data, Mann-Whitney's U-test and Spearman's rank correlation were used for statistical analysis. A significance level of 0.05 was chosen.

Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Preoperatively</th>
<th>Postoperatively</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>3 months</td>
</tr>
<tr>
<td>TSH (mU/l)</td>
<td>&lt;0.5</td>
<td>8.0*</td>
</tr>
<tr>
<td></td>
<td>(&lt;0.5-6.3)</td>
<td>(&lt;0.5-30.6)</td>
</tr>
<tr>
<td>FT4 (mU/1)</td>
<td>118</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>(32-156)</td>
<td>(34-175)</td>
</tr>
<tr>
<td>FT3 (mU/1)</td>
<td>1.8</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>(1.2-5.7)</td>
<td>(0.6-3.4)</td>
</tr>
</tbody>
</table>

*p<0.05 (Wilcoxon's test for paired differences)

Results

The 12 patients included in the study were positive both for anti-TPO and Mi-ab, whereas only 11 showed presence of Tg-ab. There was a good correlation between the semiquantitative evaluation of Mi-ab and the level of anti-TPO (Fig. 1A, R=0.82, p<0.01), but no correlation between levels of Tg-ab.

Fig. 1.

Preoperative comparative values of anti-thyroid peroxidase (anti-TPO) and microsomal antibodies (Mi-ab) (A) and anti-TPO and thyroglobulin (Tg)-ab (B) in 12 patients with thyrotoxicosis. Spearman's R: anti-TPO vs Mi-ab = 0.82, p<0.01, anti-TPO vs Tg-ab = 0.33, p>0.05.
and anti-TPO before surgery (Fig. 1B, R=0.33, p>0.1). Thyroid function tests are shown in Table 1. Five patients developed evidence of hypothyroidism 1 to 3 months postoperatively, in 4 of them the hypothyroidism was transitory, and only one needed supplementation with thyroid hormones.

The median anti-TPO level before operation was 1048 kU/l (range 68-10 517 kU/l) and fell during operation to 0.63 (range 0.37-1.28) (p<0.01) of initial concentration without further decrease during the next 1-8 h (Fig. 2). The individual values of anti-TPO before operation, and the reduction during operation, as well as fractions of initial values are shown in Table 2. Anti-TPO fell in all patients but one, with a median decrease of 227 kU/l (range 32-3098 kU/l). The decrease was correlated to the preoperative value (Table 2).

Four of the patients positive for two non-thyroid autoantibodies (ANA and IgM-RF) were positive preoperatively and their values did not fluctuate significantly either during operation or postoperatively (data not shown).

Comparatively Tg-ab decreased to 0.19 (0-0.88) of initial value. The anti-TPO level was increasing after 10 days, but did not reach initial level until between 3 and 12 months after operation. In 3 of 10 patients studied after 12 months, anti-TPO had disappeared, all 3 with low levels before operation, whereas anti-TPO was 2-4 times higher than preoperatively in 3 other patients. Tg-ab showed an earlier increase postoperatively, reaching initial concentration within 10 days. The increase at 1 year was, however, identical for both antibodies (Fig. 2). Tg-ab had disappeared in 2 patients at 1 year.

**Discussion**

Serum Tg has been shown to increase very rapidly during subtotal thyroidectomy (1,2) with a concomitant reduction in Tg-ab when present in serum preoperatively (1,3). Thyroid receptor antigens may also be released as evidenced by an acute reduction in thyrotropin binding inhibiting immunoglobulins (TBI) during operation (4), but an increase in thyroid stimulating immunoglobulins (TSI) was seen, which could be explained by a release of TSI from the thyroid gland (4,11).

TPO is the antigenic component of thyroid microsomes giving rise to Mi-ab (12), and it is present also in the thyroid cell membrane. It has previously been demonstrated that in patients operated for Graves' disease the titres of Mi-ab are

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**Table 2.**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Anti-TPO (kU/l)</th>
<th>Fractional change</th>
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</thead>
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<tr>
<td></td>
<td>Basal</td>
<td>Δ anti-TPO</td>
</tr>
<tr>
<td>1</td>
<td>6032</td>
<td>3045</td>
</tr>
<tr>
<td>2</td>
<td>1188</td>
<td>227</td>
</tr>
<tr>
<td>3</td>
<td>73</td>
<td>46</td>
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<tr>
<td>4</td>
<td>157</td>
<td>70</td>
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<tr>
<td>5</td>
<td>763</td>
<td>+212</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>32</td>
</tr>
<tr>
<td>7</td>
<td>6460</td>
<td>3098</td>
</tr>
<tr>
<td>8</td>
<td>108</td>
<td>57</td>
</tr>
<tr>
<td>9</td>
<td>908</td>
<td>122</td>
</tr>
<tr>
<td>10</td>
<td>2291</td>
<td>659</td>
</tr>
<tr>
<td>11</td>
<td>10517</td>
<td>1978</td>
</tr>
<tr>
<td>12</td>
<td>8395</td>
<td>2283</td>
</tr>
</tbody>
</table>

Spearman's R (comparing basal level with Δ anti-TPO): 0.867, p<0.0005.
rather similar to those in unoperated patients when studied years after surgery (13). This finding is compatible with the present investigation showing a return of anti-TPO values to the level before operation, except in persons with very low values who may be negative in the more insensitive Mi-ab assays.

The acute fluctuations in anti-TPO or Mi-ab during operation have been investigated previously in few patients (5,6). In one of the previous studies no modifications of the basal Mi-ab titre by passive hemagglutination were observed after thyroid surgery (6), and furthermore no immune complexes specific for Mi-ab could be detected. These results were, however in disagreement with another study on Mi-ab during thyroid surgery, in which a reduction of the level measured by enzyme-linked immunosorbent assay likewise was seen upon manipulation of the thyroid tissue (5). The present study gave evidence for an acute release of TPO into the circulation during thyroid surgery by the demonstration of decreased anti-TPO activity, although the decrease was smaller than that in Tg-ab induced by Tg. However, this does not necessarily imply that the quantitatively released amounts of the antigens Tg and TPO were different, since differences in the antigenic influence of the measurement of the antibodies might be due to different methodology (14). In one study, however, similar fractional changes were observed when employing different methods for measuring the same antibody (14). Also the antigen binding capacity of the antibodies may differ. On the other hand, Tg is present in the colloid as a storage protein apart from being present in the cell. Therefore Tg is probably more readily released into the circulation than TPO which is known to be connected with the cytoplasmic microsomes as well as being membrane bound on the thyroid cell surface (15,16). Furthermore, it cannot be excluded that also antibodies present in the thyroid gland (both Tg-ab and anti-TPO) could be released during surgery, thereby blurring the quantitative picture, as e.g. suggested for TSI (4,11).

Only a direct method for measuring TPO in serum can clarify the quantitative release of this antigen into the circulation, as previously demonstrated for Tg (1,2).

The exact nature of the released TPO antigen is unknown. However, the method for measurement of anti-TPO utilizes one monoclonal antibody (MAb 15) directed towards both a domain of TPO involved in autoantibody binding as well as the area involved in enzyme activity (17). The binding pattern of this monoclonal antibody further indicated that the epitope towards which it is directed is conformational rather than sequential (17). It could, however, not be clarified whether the released TPO was in a free enzymatic active form or attached to fragments of the thyroid membrane.

Anti-TPO has previously been shown to fluctuate during pregnancy and post partum both in women developing postpartum thyroiditis and in those who did not (18). These fluctuations were, however, more prolonged, with a gradual decrease during pregnancy and a gradual increase post partum (18,19). The fractional decrease during pregnancy was of the same order as in the operated patients in the present study, whereas the fractional increase post partum was far more pronounced than that postoperatively (13,7 vs. 1.5), indicating that completely different mechanisms were operating.

In patients with low levels preoperatively, anti-TPO remained low or disappeared postoperatively, whereas patients with high preoperative levels still had measurable or even higher concentrations 1 year after operation.

Thus TPO seems to be released into the circulation during thyroid surgery. Recent direct measurements of TPO in serum have shown measurable levels in normal persons and elevated levels in various thyroid diseases (20). Further studies are, however, needed to clarify the clinical and pathophysiological implications of this finding.

Acknowledgments

The excellent technical assistance of Lisbeth Kirkegaard and Bente Friss Mikkelsen is gratefully acknowledged. The Henning Company (Berlin) is thanked for providing the anti-TPO kits for the study. Finally, Dr Pierre Carayon is thanked for valuable discussions.

References

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