Increased Concentrations of Type IV (7 S) Collagen in Sera of Hyperthyroid Patients with Graves Disease

Teruo Shima, Yutaka Yano, Hiroyuki Goto, and Moriharu Misaki

Serum concentrations of type IV collagen (7 S) were determined in 29 patients with untreated hyperthyroidism and 30 healthy subjects. Serum 7 S collagen was significantly higher ($P < 0.0001$) in the hyperthyroid patients (6.3, SD 1.3, $\mu$g/L) than in the healthy control subjects (3.9, SD 0.6, $\mu$g/L). No difference in serum concentrations of 7 S collagen were observed between patients with normal liver function and those with abnormal liver function. Serum concentrations of 7 S collagen correlated positively with serum concentrations of free triiodothyronine ($r = 0.41$, $P < 0.05$). In the hyperthyroid patients, 7 S collagen concentrations in serum gradually fell into the normal range as thyroid function became normalized. Thus, hyperthyroidism is one of the diseases in which serum concentrations of 7 S collagen are increased.

Additional Keyphrases: thyroid function · triiodothyronine · radioimmunoassay

Type IV collagen is the major structural component of basement membrane and is synthesized by the endothelial cells (1, 2). Four molecules of type IV collagen are linked via the amino-terminal region. This disulfide-rich cross-linking domain, called 7 S collagen (3), is resistant to various proteases, including bacterial collagenase. Since the development of a sensitive immunosassay for 7 S collagen (4), measurement of 7 S collagen in serum has been used in clinical medicine as a diagnostic aid for fibrosis in primary biliary cirrhosis (5) and for chronic viral liver disease (6). During a study of serum samples from patients with Graves disease, we found that an increase in serum 7 S collagen was associated with increased concentrations of thyroid hormones. Therefore, we undertook further study to determine intra-individual changes in serum concentrations of 7 S collagen in patients during the course of altered thyroid function.

Patients and Methods

We studied 29 hyperthyroid patients with Graves disease: 4 men and 25 women (mean age 36 years, range 15–60 years). These patients were divided into two groups according to liver function: 18 had normal liver function, 11 had abnormal liver function. Liver-function tests included assays of aspartate aminotransferase (EC 2.6.1.1) and alanine aminotransferase (EC 2.6.1.2) in serum. No patients were taking drugs other than anti-thyroid drugs, and none had any disease other than Graves disease and abnormal liver function.

Sixteen were also studied after returning to euthyroid status after treatment with either methimazole or propylthiouracil. Blood samples were obtained from these patients at 1-month intervals to establish how much time they required to become euthyroid. Euthyroid was defined as having concentrations of free triiodothyronine (FT$_3$) and of thyrotropin (TSH) within the respective normal ranges of 2.5–5.5 ng/L and 0.5–5.2 milli-international units/L (mIU/L).$^2$

Informed consent was obtained from each subject. Control serum samples were obtained from 30 volunteers, 4 men and 26 women (mean age 36 years, range 18–59 years).

Blood samples were obtained between 0800 and 1100, and serum was stored at $-20^\circ$C until analysis.

Concentrations of 7 S collagen were determined with a double-antibody radioimmunoassay kit (Nippon DPC Corp., Tokyo, Japan) in which $^{125}$-labeled 7 S domain of type IV collagen isolated from human placenta is the tracer, rabbit antiserum against this material is the primary antibody, and goat anti-rabbit IgG antiserum is the secondary antibody (6). The pH 7.4 buffer solution used in the RIA contains, per liter, 0.05 mol of sodium phosphate, 0.15 mol of sodium chloride, and 1 g of bovine serum albumin. The detection limit of the assay for 7 S collagen is 1.8 $\mu$g/L, based on the concentration at 95% of maximum binding, determined from the results for 50 zero-calibrator samples.

Intra-assay and interassay coefficients of variation were 6.6% (n = 10) and 5.0% (n = 20), respectively.

Serum FT$_3$ and free thyroxine (FT$_4$) were measured with commercial radioimmunoassay kits (Daichii Radioisotopes Corp. and Eiken Corp., both in Tokyo, Japan). Serum TSH concentrations were measured with a sensitive immunoradiometric method, with a lower detection limit of 0.1 mIU/L (Daichii Radioisotopes Corp.). Serum TSH-binding-inhibiting immunoglobulin (TBI2) activity was measured with a Smith kit (Baxter Healthcare Corp., Cambridge, MA).

Throughout, results are expressed as mean ± SD. The statistical significance of the differences between the means was assessed by using the Wilcoxon test. Comparisons between effects on various results were tested by linear-regression methods.

Results

Serum thyroid hormone concentrations and alanine aminotransferase values from 29 hyperthyroid patients

$^2$ Nonstandard abbreviations: FT$_3$, free triiodothyronine; FT$_4$, free thyroxine; TSH, thyrotropin; and TBI2, TSH-binding-inhibiting immunoglobulin.
Table 1. Clinical Data from Patients with Untreated Hyperthyroidism

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Age, years</th>
<th>FT$_{3}$ ng/L</th>
<th>FT$_{4}$ ng/L</th>
<th>TBII, %</th>
<th>ALT, U/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated hyperthyroidism</td>
<td>29</td>
<td>36 ± 13</td>
<td>16.2 ± 7.0</td>
<td>55 ± 25</td>
<td>42.3 ± 26.0</td>
<td>40 ± 38</td>
</tr>
<tr>
<td>With normal liver function</td>
<td>18</td>
<td>32 ± 13</td>
<td>15.5 ± 6.8</td>
<td>55 ± 27</td>
<td>41.2 ± 26.9</td>
<td>21 ± 6</td>
</tr>
<tr>
<td>With abnormal liver function</td>
<td>11</td>
<td>41 ± 11</td>
<td>17.3 ± 7.5</td>
<td>53 ± 23</td>
<td>44.0 ± 26.0</td>
<td>71 ± 48</td>
</tr>
<tr>
<td>Healthy control subjects</td>
<td>30</td>
<td>36 ± 14</td>
<td>4.0 ± 0.7</td>
<td>16 ± 3</td>
<td>—</td>
<td>15 ± 10</td>
</tr>
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ALT, alanine aminotransferase.

![Graph](image-url)

Fig. 1. Type IV collagen (7 S) concentrations in serum from normal control subjects and untreated hyperthyroid patients with normal and abnormal liver function

Abnormal liver function reflected by "elevated SGPT" (increased alanine aminotransferase)

Discussion

The significantly increased 7 S collagen values found in serum from the hyperthyroid patients in this study suggest that 7 S collagen metabolism is related to thyroid hormone availability. This hypothesis was also supported by the observed decline in serum 7 S collagen concentrations of these patients during successful thyr-rostatic treatment.

Increased values of 7 S collagen are found in patients with primary biliary cirrhosis (5) and chronic viral liver disease (6). The concentrations of 7 S collagen in serum of patients with chronic active hepatitis and chronic active hepatitis with lobular disorganization were 5.3 (SD 1.7) µg/L and 8.1 (SD 2.1) µg/L, respectively. Serum 7 S collagen concentrations correlated with the grade of fibrosis of liver specimens. Klion et al. (7) reported that biopsy specimens of liver from hyperthyroid patients...
were similar and showed a nonspecific reactive hepatitis. Many portal tracts showed mild infiltration of mononuclear cells, mainly lymphocytes. The limiting plate of the parenchyma was intact, and hepatocellular steatosis was moderate. Bundles of collagen representing the normal stroma were not increased in the space of Disse.

The histological liver changes in Graves disease are so minor that they cannot account for the alterations in the serum concentrations of 7S collagen. In the present study, results of nonspecific liver tests, such as those measuring alanine aminotransferase concentrations, were abnormal in 38% of the patients. However, no difference in serum concentrations of 7S collagen was found between hyperthyroid patients with abnormal liver function and those with normal function. All this suggests that pathological changes in the liver of patients with thyrotoxicosis did not influence serum concentrations of 7S collagen. Increased serum values of 7S collagen have also been found in patients with diabetes mellitus (8). These values did not change during short periods of blood glucose control (9). The present study included no cases complicated with diabetes mellitus.

Type IV collagen is synthesized by the endothelial cells (1, 2). It is the major protein in the isolated subendothelial matrix and a minor component in the medium protein, as measured by densitometric analysis of the autoradiograms of secreted protein in sodium dodecyl sulfate gels (2). Endothelial cells are metabolically active, synthesizing a multitude of proteins such as angiotensin-converting enzyme (10), fibronectin (11), and factor VIII-related antigen (12). Increased values of plasma angiotensin-converting enzyme (13–15), fibronectin (15, 16), and factor VIII-related antigen (15, 17) have been found in hyperthyroid patients. The mechanism responsible for the hyperthyroidism-associated increase in 7S collagen concentration remains unknown, but could involve increased synthesis and (or) turnover of type IV collagen in the tissue. Because the degradation of many hormones and other substrates is increased in hyperthyroidism, the increase in 7S collagen in serum is probably also due to increased degradation of tissue. On the other hand, increases in plasma concentrations of endothelium-associated proteins such as angiotensin-converting enzyme, fibronectin, and factor
VIII-related antigen in hyperthyroidism (15) are assumed to be due to their increased synthesis. Type IV collagen synthesized in the endothelial cells may similarly overflow into the bloodstream.

References