Clinical Evaluation of a Hemagglutination Method for Microsomal and Thyroglobulin Antibodies in Autoimmune Thyroid Disease*

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ABSTRACT

A simple and reproducible hemagglutination technique for detecting microsomal and thyroglobulin antibodies has been evaluated in normal subjects and in patients with thyroid disease. Microsomal antibodies were detected in 89 percent of all patients with autoimmune thyroid disease and in 97 percent of patients with biopsy proven Hashimoto's thyroiditis. In contrast, thyroglobulin antibodies were present in only 55 percent of patients with biopsy proven Hashimoto's thyroiditis and in 44 percent of all patients with suspected autoimmune thyroid disease. It is suggested, therefore, that measurement of microsomal antibodies by a simple hemagglutination technique be carried out in all patients with suspected thyroid disorders.

Introduction

Autoimmune thyroid disease is usually associated with circulating antibodies against subcellular and colloid components of the thyroid, including thyroglobulin and microsomes. The tanned sheep red cell agglutination test to detect thyroglobulin antibody is most commonly used to diagnose Hashimoto's or autoimmune thyroiditis. It is rapid and reproducible but not sufficiently sensitive to diagnose this disorder consistently.\(^3,6,12\) In contrast, antibodies to thyroid microsomal (cytoplasmic) anti-

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tibodies were more frequently detected in patients with autoimmune thyroid disease than were thyroglobulin antibodies.

Materials and Methods

Studies were carried out in the sera of 27 patients with biopsy proven Hashimoto's thyroiditis, 27 patients with clinically probable Hashimoto's thyroiditis, 25 patients with spontaneous hypothyroidism, 22 patients with nontoxic goiter, 21 patients with thyrotoxic Graves' disease, and 85 normal volunteers. Hyperthyroidism and hypothyroidism were confined by appropriate thyroid function tests.

The microsomal hemagglutination test (MHT) and the thyroglobulin hemagglutination test (THT) were carried out by the method of Fujita et al. Details of the test procedure are carefully outlined in the kits. Serum dilutions were carried out from 1:20 through 1:160,000. The serial dilutions were mechanically carried out by the use of a "Handititer." In some patients, microsomal (MFAB) and colloid (CFAB) antibodies were also determined by an immunofluorescent technique as previously described.

Results

Evaluation of the Tanned Red Cell Techniques

Tanned red cell tests for detecting microsomal and thyroglobulin antibodies in the sera of 27 subjects were carried out on two occasions. All negative tests remained negative and positive tests were identical or varied by only one dilution when tested for the second time. In a few sera with moderate or high titers, low dilutions (1:20 to 1:320) were negative and higher dilutions became positive. It is, therefore,

necessary to carry out the hemagglutination test through high dilutions in order to avoid false negative tests. Further studies to characterize this "prozone" or "blocking phenomenon" were not carried out.

Comparison of the MHT and MFAB Tests

Both tests for detecting microsomal antibodies were carried out in 44 normal subjects, 31 patients with Hashimoto's thyroiditis, 15 patients with primary hypothyroidism and 18 patients with Graves' disease. Two normal subjects had low titers by MHT (1:40) but were negative by MFAB, whereas one normal subject was positive by both tests. The remaining normal subjects were negative by both tests. In 20 patients with biopsy proven Hashimoto's thyroiditis, all had high titers by the hemagglutination test (100 percent positive). MFAB was positive in 17 of these patients (85 percent positive). In 11 patients with probable Hashimoto's thyroiditis, microsomal antibodies were detected by both methods in all patients. In the 15 patients with primary hypothyroidism, microsomal antibodies were absent by both methods in three, positive by both methods in ten, and positive by MHT and negative by MFAB in two. Finally, in the 18 patients with Graves' disease, MHT was positive in 17 (94 percent) and MFAB positive in 13 (72 percent).

Colloid antibodies were also evaluated by the immunofluorescent technique in these patients. CFAB was positive in 25 percent of the normal subjects, suggesting a high incidence of false positive tests. CFAB was positive in 90 percent of the patients with biopsy proven Hashimoto's thyroiditis, in 91 percent of the patients with probable Hashimoto's thyroiditis, in 52 percent of patients with primary hypothyroidism and in 66 percent of patients with Graves' disease.

* Manufactured by Fujizoki Pharmaceutical Co., LTD and supplied by Ames Co., Elkhart, IN.
† Supplied by Ames Co., Elkhart, IN.
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**Figure 1.** Hemagglutination test for microsomal antibodies (MHT) in normal subjects and in patients with thyroid disease. Solid dots represent individual subjects. The numbers in the bars represent the number of subjects with negative tests.

**HEMAGGLUTINATION TEST FOR MICROSOMAL AND THYROGLOBULIN ANTIBODIES IN PATIENTS WITH VARIOUS THYROID DISORDERS**

Thyroid antibodies (MHT or THT) were detected in only four of 85 normal subjects. Microsomal antibodies were present in high titer (1:160 or greater) in 26 of 27 patients with biopsy proven Hashimoto's thyroiditis (97 percent) whereas thyroglobulin antibodies were detected in only 15 of these patients (55 percent). Microsomal antibodies were present in high titer in 25 of 27 patients with probable Hashimoto's thyroiditis. In contrast, thyroglobulin antibodies were detected in only 18 percent of these patients. In 25 patients with primary hypothyroidism, microsomal antibodies were present in 84 percent and thyroglobulin antibodies in 64 percent. In the patients with Graves’ disease, 80 percent had microsomal and 38 percent had thyroglobulin antibodies. A far smaller proportion of the 22 patients with non-toxic goiter had positive antibodies (27 percent microsomal and 5 percent thyroglobulin). Although the possibility of autoimmune thyroid disease in these patients with non-toxic goiter cannot be definitely ruled out, the thyroid glands did not have the clinical characteristic of Hashimoto’s thyroiditis and the titers were low in all except one patient.

It should be pointed out that in patients with proven or suspected thyroid autoimmune disease (Hashimoto’s thyroiditis, primary hypothyroidism and Graves’ disease), the microsomal antibody titer was almost always 1:160 or greater in those patients with positive tests.

**Discussion**

The occurrence of antibodies in serum against various subcellular fractions of the thyroid has been a useful marker for the diagnosis of autoimmune thyroid disease, especially Hashimoto’s thyroiditis. Thyroglobulin antibodies are usually detected by the tanned sheep red cell hemagglutination technique which is readily available in most clinical laboratories. Immunofluorescent techniques for detecting cytoplasmic antibodies are more difficult to carry out, more subjective in their inter-
TABLE I

Microsomal and Thyroglobulin Antibodies in Sera of Normal Subjects and Patients with Thyroid Disease

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Hemagglutination Test*</th>
<th>Immunofluorescent Test+</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MHT</td>
<td>THA</td>
</tr>
<tr>
<td></td>
<td>Total No.</td>
<td>85</td>
</tr>
<tr>
<td></td>
<td>No. Positive</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>% Positive</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>44</td>
</tr>
<tr>
<td>Biopsy Proven</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Hashimoto's</td>
<td>Total No.</td>
<td>26</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>No. Positive</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td>% Positive</td>
<td>3</td>
</tr>
<tr>
<td>Probable</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>Hashimoto's</td>
<td>Total No.</td>
<td>25</td>
</tr>
<tr>
<td>Thyroiditis</td>
<td>No. Positive</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>% Positive</td>
<td>100</td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>
| Hypothyroidism             | *A positive test ranges from weakly to strongly positive fluorescent staining. A doubtful test is classified as negative. Microsomal hemagglutination test = Thyroglobulin hemagglutination test Microsomal fluorescent antibody test = Colloid fluorescent antibody test

A positive test represents agglutination of red blood cells at a titer of 1:20 or more.

Fujita et al. have devised a hemagglutination method for detecting microsomal antibodies by sensitizing sheep red cells with human microsomal rather than thyroglobulin antigen. This test is simple and reproducible and, in the present study, somewhat more sensitive than the immunofluorescent technique in patients with autoimmune thyroid disease. These findings are in agreement with those reported by other workers, but not all.

The MHT was positive at a titer of 1:160 or more in 97 percent of patients with autoimmune thyroiditis, a frequency which is similar to that reported by Aoki and Amino and their coworkers. This high frequency of microsomal antibodies in the sera of patients with Hashimoto’s thyroiditis is greater than that reported by others using immunofluorescent techniques, but similar to that found by a competitive binding radioassay. The high incidence of positive MHT in Graves’ disease and primary hypothyroidism is not unexpected. Graves’ disease is included in the spectrum of autoimmune thyroid disorders and may coexist with Hashimoto’s disease.

Autoimmune thyroiditis is often the underlying cause of primary hypothyroidism and, prior to the availability of serum TSH determinations, a positive antibody titer was often used to distinguish between primary and secondary hypothyroidism.

Although the MHT was positive in 6 of 22 patients with non-toxic goiter which clinically was not thyroiditis, the titer was almost always less than 1:160. Hashimoto’s thyroiditis could have been present in at least some of these glands.
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tibodies. This low frequency may not be due to false positive tests but might reflect early autoimmune thyroid disease since the incidence of thyroid disease in the general population has been estimated to be approximately 5 percent.1,6,9

In contrast to the high frequency of microsomal antibodies in the sera of patients with autoimmune thyroid disease, thyroglobulin antibodies were detected by the hemagglutination technique in only 43 percent of all patients with Hashimoto’s thyroiditis, primary hypothyroidism and Graves’ disease. The low incidence of 35 percent positivity in all patients with Hashimoto’s thyroiditis was even lower than that reported previously in this disorder,3,7,12 but confirms the observation that the standard red cell hemagglutination test for detecting thyroglobulin antibodies in patients with suspected autoimmune thyroid disease is not a useful screening test in view of the large proportion of false negative tests. Furthermore, the THT was positive with a negative MHT in only two patients with probable autoimmune thyroid disease.

Recently, a radioimmunoassay for anti-thyroglobulin antibodies was described.8,11 The sensitivity of this assay is reported to be 100 percent in detecting thyroglobulin antibodies in surgically proven Hashimoto’s thyroiditis. The enhanced sensitivity of this method for detecting thyroglobulin antibodies represents a significant improvement over current methods. However, since a radioimmunoassay technique is involved, its practical application in a clinical setting is as yet unknown.

It has been suggested2 that the greater sensitivity of the microsomal hemagglutination test might be due to the detection of heterogenous antibodies, including thyroglobulin antibody, since the microsomal antigen used to coat the red cells is prepared from hyperfunctioning thyroidal follicular cells from Graves' disease thyroids. In the present study, however, it is extremely unlikely that cross reactivity of thyroglobulin antibody in the microsomal test could explain the greater sensitivity of the latter assay. Forty-two sera were negative for thyroglobulin antibody and positive for microsomal antibody, and 3 sera were strongly positive for thyroglobulin antibody and negative for microsomal antibody.

The present findings suggest that the tanned red cell method for detecting thyroglobulin antibodies is not a useful screening procedure for diagnosing autoimmune thyroid disease in contrast to the efficacy of the microsomal antibody test. A negative or weakly positive (< 1:160) microsomal antibody titer is strong evidence against a diagnosis of Hashimoto’s thyroiditis. It must be emphasized that a positive test is not necessarily diagnostic of chronic thyroiditis since microsomal antibodies are often detected in other autoimmune thyroid disorders, including Graves’ disease.

Acknowledgments

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References


