

CLINICAL AND IMMUNOLOGICAL ASPECTS IN HASHIMOTO'S THYROIDITIS

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ABSTRACT

We propose to establish the relationship between the clinical, functional and immunological parameters in a cohort of women with Hashimoto's thyroiditis. The study included 68 patients between 19 and 70 who were hospitalized for two years in the Endocrinology Clinic of the Hospital in Timisoara. The thyroidal dimensions and the aspects of the thyroidal stroma were shown by echography examinations. The functional parameters like the serum TSH and free T4 concentrations were determined by chemiluminescent methods.

The immunological investigations (antithyroglobulin and antiperoxidase antibodies) were performed by the ELISA method. Comparisons were performed by the t test and the Pearson correlation analysis. The correlation between the aspects of thyroidal stroma and thyroid function revealed hypothyroidism in most cases with moderate (++) or severe (+++) echogenity.

Another researched aspect was that of correlation of the serum levels of TSH with the thyroidal dimension. The following correlations were obtained:

$r = -0.14$ in patients with clinical hypothyroidism and
 $r = -0.3055$ in those with subclinical hypothyroidism.

The Pearson coefficients for the level of TSH in μm and those of the antiperoxidase antibodies, according to clinical and functional categories, revealed the following results:

$r = -0.1580$ in patients with clinical hypothyroidism and
 $r = -0.1961$ in those with subclinical hypothyroidism.

Patients with clinical hypothyroidism have significant higher serum level of TSH as compared with those of the patients with subclinical hypothyroidism ($p=0.0001$). We established a relation between the severe hypoechogenity and hypothyroidism status in patients with positive serum antibodies ($r=0.5695$).

INTRODUCTION

Hashimoto's thyroiditis is an inflammatory disorder of unknown aetiology, which results in progressive destruction of the thyroid gland. It is found most commonly in middle – aged and young females, but it also occurs in other age groups, including children, in whom it may cause goitre. Although it is distributed throughout the world without racial and ethnic restriction, it occurs more commonly in families where another member has an autoimmune thyroid disease. It is observed in conjunction with Grave's disease in a form of autoimmune–overlap syndrome 28.

Hashimoto's thyroiditis is primarily associated with symptoms of altered thyroid function. Early in the course of the disease the patient is usually euthyroid, but may experience clinical hyperthyroidism due to the inflammatory breakdown of thyroid follicles with release of thyroid hormones. In contrast, late in the disease the patient is often hypothyroid because of progressive destruction of the thyroid gland. The most common eventual outcome of Hashimoto's disease is hypothyroidism 3, 5.

Diagnosis involves two considerations: the differential diagnosis of the thyroid lesion and the assessment of the metabolic status of the patient. A diffuse, firm goitre with pyramidal lobe enlargement, and without signs of thyrotoxicosis, should suggest the diagnosis of Hashimoto's thyroiditis. Most often the gland is bosselated or "nubbey". It is usually symmetrical, although much variation in symmetry (as well as consistency) can occur. The association of goitre with hypothyroidism is almost the condition of the diagnosis, but it is also seen in certain syndromes due to defective hormone synthesis or hormone response. Most commonly the gland is two to four times the normal size 2, 29.

Multinodular goitre occurs in significant incidence in adult women; thus the co-occurrence of multinodular goitre and Hashimoto's thyroiditis is not rare, and may provide the finding of a grossly nodular gland in a patient who is mildly hypothyroid and positive antibody tests. The concentration of T4 and of FT4 range from low to high but are most typically in the normal or low range 13.

Gamma-globulin levels may be elevated, although usually they are normal. This alteration evidently reflects the presence of high concentrations of circulating antibodies to thyroglobulin (Tg), for an antibody concentration as high as 5.2 mg/ml has been reported 13. T4 and FT4 are normal or low. The serum TSH concentration reflects the patient's metabolic status. However, some patients are clinically euthyroid, with normal FT4 and T3 levels, but have mildly elevated TSH. Whether this "subclinical hypothyroidism" represents partial or complete compensation is a matter of debate. Anti-thyroid peroxidase antibody and, less frequently, anti-thyroglobulin antibody, are present in serum. High levels are diagnostic of autoimmune thyroid disease. Anti-thyroglobulin antibody is positive in about 80% of patients, and if both anti-thyroglobulin and anti-thyroid peroxidase antibodies are measured, 97% are positive. Young patients tend to have lower or occasionally negative levels. In this age group, even low titres signify the presence of thyroid autoimmunity 13, 29.

Ultrasound may display an enlarged gland with normal texture, a characteristic picture with very low echogenicity, or a suggestion of multiple ill-defined nodules 20, 29.

OBJECTIVE

Our aim is to establish the relationship between the clinical, functional and immunological parameters in a cohort of women with Hashimoto's thyroiditis.

MATERIALS AND METHODS

The study included 68 patients between 19 and 70 who were hospitalised for two years in the Endocrinology Clinic of the County Clinical Hospital No 1 Timisoara. The thyroid dimensions and the aspects of the thyroid stroma were shown by echography examinations. Blood samples were obtained for the measurement of thyroid antibodies as well as serum TSH and free T4 concentration and stored at below -20°C before the assay.

The functional parameters like the serum TSH and free T4 concentrations were determined by the chemiluminescent methods 25. The Vitros TSH assay is performed by using the Vitros TSH Reagent Pack and Vitros Immunodiagnostic Products TSH Calibrators on the Vitros Immunodiagnostic System. An immunometric technique is used; this involves the simultaneous reaction of TSH present in the sample with a biotinylated antibody (mouse monoclonal anti-whole TSH) and a horseradish peroxidase (HRP)-labelled antibody conjugate (mouse monoclonal anti-TSH α -subunit). The antigen-antibody complex is captured by streptavidin on the wells; unbound materials are removed by washing.

The bound HRP-conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivate and a peracid salt) and an electron-transfer agent are added to the wells. The HRP in the bound conjugate catalyses the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of the produced light and prolongs its emission. The amount of bound HRP-conjugate is directly proportional to the concentration of TSH present.

A normal reference interval of 0.465 to 4.68 mIU/L with a mean of 1.48 mIU/L was obtained from patients of euthyroid status 15, 16.

The Vitros Free T4 assay is performed using the Vitros Free T4 Reagent Pack and Vitros Immunodiagnostic Products Free T4 Calibrators on the Vitros Immunodiagnostic System. A direct, labelled-antibody, competitive-immunoassay technique is used. FT4 present in the sample competes with ligand on the modified well surface for a limited number of binding sites on a horseradish peroxidase (HRP)-labelled antibody conjugate (sheep

anti-T4). The well surface has been modified to act as a ligand for uncombined conjugate. Unbound materials are removed by washing. The assay design, with optimal reagent concentrations, ensures that disturbance of the T4/binding protein equilibrium is so small as to be negligible.

The bound HRP conjugate is measured by a luminescent reaction. A reagent containing luminogenic substrates (a luminol derivative and a peracid salt) and an electron-transfer agent are added to the wells. The HRP in the bound conjugate catalyses the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of the produced light and prolongs its emission. The light signals are read by the Vitros System. The amount of bound HRP conjugate is indirectly proportional to the concentration of FT4 present.

The following reference interval (1.0 and 99.0 percentiles) was determined: a FT4 reference interval of 10.0 to 28.2 pmol/L with a median of 15.9 pmol/L was obtained from apparently euthyroid patients, not on treatment 17,18 . The immunological investigations (anti-thyroglobulin and antiperoxidase antibodies) were performed by the ELISA method.

The Kallestad Tg (TPO) Assay included microwells that are coated with purified thyroglobulin (thyroid peroxidase) antigen. During the first incubation, specific autoantibodies in diluted serum will bind to the antigen coating. The microwells are then washed to remove unbound serum components. A conjugate of enzyme-labelled monoclonal antibody to human IgG binds to surface-bound antibodies in the second incubation. After a further washing step, specific antibodies are traced by incubation with substrate solution. Addition of stopping solution terminates the reaction and provides the appropriate pH for colour development. The intensity of the resultant magenta colour is read spectrophotometrically.

In the quantitative procedure, the amount of conjugate bound in the presence of the unknown serum or plasma sample can be interpolated from a dose-response curve. This curve is based on standards that are calibrated against the International Reference Preparation of anti-thyroglobulin or anti-thyroid peroxidase.

Each Kallestad Tg (TPO) Assay is to be considered separately when calculating and interpreting results. The negative concentration must be lower than 100 IU/ml for Tg and 50IU/ml for TPO. The concentration of the positive control must fall within the range printed on the positive control label.

The mean absorbance value of each calibrator was calculated and plotted against log calibrator concentration on suitable graph paper. The concentrations of the samples can then be read from the calibrator curve.

| Calibrator | IU/ml |
|------------|-------|
| S0 | 0 |
| S1 | 100 |
| S2 | 400 |
| S3 | 1500 |
| S4 | 5000 |

Table 1. Anti-Tg Calibrators

| Calibrator | IU/ml |
|------------|-------|
| S0 | 0 |
| S1 | 50 |
| S2 | 250 |
| S3 | 900 |
| S4 | 3000 |

Table 2. Anti-TPO Calibrators

Samples that give absorbances higher than the top calibrator are out of range of this assay and should be stated as 5000 IU/ml (3000IU/ml). Such samples may be diluted and a further 1/10 dilution is recommended. When calculating results for any sample that has been further diluted, the obtained sample concentration must be corrected by the dilution factor 21, 22.

Comparisons of calculated data were performed by the t-test and the Pearson correlation analysis.

RESULTS

The mean and standard deviation of clinical, functional and immunological parameters are shown in the following table.

| | Euthyroid status | Subclinical hypothyroidism | Clinical hypothyroidism | Hyperthyroidism status |
|-------------------|--------------------|----------------------------|-------------------------|------------------------|
| Number of cases | 23 | 14 | 27 | 4 |
| TSH | 4.07±3.68 | 18.23±11.04 | 46.64±32.03 | 0.03±0.01 |
| FT4 | 16.69±2.54 | 13.03±2.38 | 5.24±2.38 | 41.34±8.26 |
| Thyroid volume | 18.67±10.83 | 28.06±9.84 | 18.38±18.28 | 19.50±11.31 |
| Anti-TPO antibody | 929.21± 2265.65 | 1512.51± 2666.14 | 846.14± 1745.49 | 441.95± 447.11 |
| Anti-Tg antibody | 367.35± 253.83 | 317.47± 316.20 | 188.54± 226.28 | 149.85± 100.1 |
| Age | 35.25±14.80 | 42.23±14.11 | 48.03±11.51 | 27.33±15.37 |

Table 3. The clinical, functional and immunological parameters in Hashimoto's thyroiditis

| Thyroidal volume (ml) | Number of cases | Number of cases with echogenity | | |
|-----------------------|-----------------|---------------------------------|----|-----|
| | | + | ++ | +++ |
| 16.7-19.9 | 8 | 1 | 4 | 3 |
| 20.0-29.9 | 13 | 2 | 7 | 4 |
| 30.0-39.9 | 4 | | 2 | 2 |
| 40.0-49.9 | 1 | | | 1 |
| 50.0-59.9 | 3 | | | 3 |
| 60.0-69.9 | 1 | | | 1 |

Table 4. The distribution of cases according to the thyroidal volume and the degree of echogenity

Another researched aspect was that of the correlation of the serum levels of the TSH with the thyroidal dimension.

The following correlations were obtained:

- $r = + 0.3942$, a moderate direct correlation in patients with euthyroid;
- $r = - 0.14$, an inverse insignificant correlation in patients with clinical hypothyroidism $r = - 0.3055$, a moderate inverse correlation in those with subclinical hypothyroidism.

The Pearson coefficients for the TSH serum and those of the antiperoxidase antibodies according to the clinical and functional categories revealed the following results:

- $r = - 0.1034$ in patients with euthyroidism;
- $r = - 0.1580$ in patients with clinical hypothyroidism;
- $r = - 0.1961$ in those with subclinical hypothyroidism.

The anti-thyroglobulin antibody has had positive values only in 3 of 23 % of patients with euthyroidal function. The Pearson coefficient of the correlation between the serum level of TSH and the titres of anti-thyroglobulin antibody has the following values:

- $r = 0.3521$ in patients with clinical hypothyroidism;
- $r = - 0.1130$ in patients with subclinical hypothyroidism.

The patients with clinical hypothyroidism have significant higher serum level of TSH when compared with

those of the patients with subclinical hypothyroidism ($p=0.0001$).

We didn't obtain a significant difference between the serum levels of anti-TPO and anti-Tg antibodies when we compared the clinical and subclinical stages of the disease ($p = 0.6385$ for anti-TPO and $p = 0.2083$ for anti-Tg).

DISCUSSION

Medical literature has revealed that the diffuse Hashimoto's goitre has a peculiar firm consistency like rubber; the goitre may regress with time but can persist in many cases 7. In some instances the patients present an initial transient hyperthyroid stage, called Hashitoxicosis. The term Hashimoto's disease is generally used to indicate auto-immune destruction of thymocytes which may eventually result in hypothyroidism although many cases remain euthyroid 29. Usually, the thyroid is diffusely enlarged, including the pyramidal lobe, with variable size (from minimal to massive), of firm or hard consistency, irregular surface and progressive growth, according to the literature information 14, 24.

In this study only 4 to 68 percent of cases have hyperthyroidal function, and this is in relation with other medical studies in which the hyperthyroidism occurs as the initial manifestation of autoimmune disease in less than 5 percent of cases.

According to Dayan's studies, the risk of a progression to overt hypothyroidism was five times higher among men than among women and increased markedly with age in 45-year old women or older. Other studies revealed the following aspects: in community surveys in Detroit 1, Baltimore 10, and Whickham 26 and a survey of general practices in Birmingham, United Kingdom 18, 27, 8 to 17 percent of subjects older than 55 to 60 had subclinical hypothyroidism (a higher serum thyrotropin concentration than 5 mU per liter and a normal serum thyroxine concentration), and 3 to 7 percent had serum thyrotropin values higher than 10 mU per liter 1, 23. Despite these high figures, the prevalence of overt hypothyroidism (high serum thyrotropin and low serum thyroxine concentrations) was only 1.4 percent in women in Whickham and only about 3 percent in elderly women in Framingham, Massachusetts 23.

In the present study, 14 to 68 percent women have had subclinical hypothyroidism (TSH between 18.23 ± 11.04 mIU/ml, above upper normal limit, and Free T4 between 13.03 ± 2.38 pmol/ml, within normal reference range) and 12 of 68 percent with overt hypothyroidism (TSH higher than 66.7 mIU/ml and FT4 lower than 2.91 pmol/l).

Ultrasonography has shown an enlarged thyroid gland with a diffusely hypoechogenic pattern in 18 to 77 percent of patients, but the findings are not specific, according to Dayan's studies. 4, 14, 24 Marcocci revealed a normal or a discreet hypoechogenicity in patients with euthyroidism status, but in hypothyroidism a moderate (++) and severe (+++) echogenicity are shown, according to our data. 12 According to Kasagi's study anti-Tg and anti-TPO were positive in 61 of 83 percent of patients with Hashimoto's thyroiditis, 3 of 83 percent were negative for both and 19 of 83 percent were positive for anti-Tg alone. 8 In the present study, the anti-Tg and anti-TPO antibodies were positive in 23 of 68 percent of cases with hypothyroidism and severe hypoechogenicity of thyroidal parenchyma. Six of 23 percent cases with positive antibodies have had overt hypothyroidism status. In 35 of 68 percent of cases the anti-Tg antibody were detectable in serum.

CONCLUSIONS

- The correlation between the aspects of the thyroidal stroma and the thyroidal function has revealed hypothyroidism with moderate (++) and severe (+++) echogenicity in most cases.
- We have established a direct relation between the severe hypoechogenicity and hypothyroidism status in patients with positive serum antibodies ($r = 0,5695$).
- That aspect could be a prognostic marker in the evolution of the hypothyroid status in Hashimoto's thyroiditis.

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