Increase in Plasma Thyrotropin Levels in Hypothyroid Patients during Treatment due to a Defect in the Commercial Preparation

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ABSTRACT

Around mid-1995, the Molecular Endocrinology Laboratory of the Regional Hospital (Malaga, Spain) began detecting an increase in TSH levels in the serum of patients under study to control the treatment of hypothyroidism with levothyroxine. Over a period of 5 months, of a total of 467 hypothyroid patients treated with Levothroid, 53% had TSH levels higher than 6 μU/mL. The reliability of the biochemical results was verified by duplicating 56 randomly chosen samples from all those with high TSH levels and by an external control performed in four different laboratories. The amount of levothyroxine present in the tablets was analyzed by RIA, high performance liquid chromatography, and their iodine contents. The lowest levels of levothyroxine found in the 50-μg Levothroid tablets were those determined by RIA, with a mean value of 32.3 μg, resulting in a 35.3% loss of activity. The mean value of levothyroxine found in these same tablets by high performance liquid chromatography was 39.3 μg, amounting to a 21.3% loss in activity. The iodine showed no significant loss in these tablets, with a mean experimental value of 48 μg. The commercial laboratory withdrew lot J from the market, the one in which these deficiencies were found. (J Clin Endocrinol Metab 82: 3192–3195, 1997)

The appearance of synthetic preparations of levothyroxine in the 1950s together with the development of highly sensitive and specific techniques for the measurement of TSH seemed to enable easy control of thyroid function in hypothyroidism, but it was later found that a series of factors could make maintenance of the euthyroid state difficult (1, 2). Apart from individual differences regarding intestinal absorption of the preparations (3), interference has been reported with other medicines (2) or with the consumption of fiber (4), and autoantibodies against thyroid hormones have been detected (5). Divergence between the amount of active ingredient specified on the container and the true concentration in the tablets to be taken by the patients has also been reported (6), leading to added dosage problems, resulting in modifications in circulating hormone levels, especially TSH (7). Similar complications have occasionally appeared with the manufacture of new presentations of the preparation or when there has been a reformulation (3, 8), although a change in brand does not necessarily result in problems (9).

When over 50% of patients treated with the same preparation of levothyroxine present elevated TSH levels, even in the absence of clinical signs, it is necessary to analyze the reasons for this change, which obviously has risks for the patients (1–3). In this case it is the hormone assay that can provide the alarm signal and that requires an adequate working strategy and assay system to determine the precise levels of TSH and T4 (1).

Around the middle of 1995, the Molecular Endocrinology Laboratory of the Regional Hospital (Malaga, Spain) began to detect an increase in TSH levels in the serum of patients under study to control the treatment of hypothyroidism with levothyroxine. To safeguard the laboratory from any possible responsibility, a retrospective analysis was performed, and the biochemical thyroid function levels were followed in those patients requiring periodic study to control their treatment, with some results randomly duplicated with an alternative technique. This study lead to the confirmation of raised TSH levels in those hypothyroid patients treated with Levothroid.

We present the results of the biochemical evaluation of treatment with Levothroid in 467 patients belonging to four etiological groups [adult hypothyroidism (AH), thyroid cancer (TC), congenital hypothyroidism (CH), and pregnancy-associated hypothyroidism (PH)] as well as data concerning analysis of the content of levothyroxine and iodine in the tablets corresponding to different pharmaceutical preparations.

Subjects and Methods

Over a period of 5 months, we studied 267 AH (160 women and 107 men), 84 CH (57 women and 27 men), 108 TC (88 women and 20 men), 8 PH, and a control group composed of 140 people with normal TSH levels (84 women and 56 men). All patients received substitute treatment with levothyroxine sodium (Levothroid, Rhone-Poulenc Rorer, Paris,
France) and were attended in the Molecular Endocrinology Laboratory by the same specialist (M.J.G.), who carried out a short interview before the extraction of blood to ascertain any medications being taken and any other data that could be of interest in the biochemical determination of the results and for the preparation of the laboratory report to be sent to the attending specialist. In the treatment controls, longitudinal follow-up of the most significant parameters was compulsory. The sequence of the biochemical studies was determined by clinical protocols (1, 2, 10, 11).

In Spain there are only two presentations for Levothroid tablets (50 and 100 μg), so that fractionated dosages are difficult, especially in children. The treatment followed by our patients is extremely varied. Patients with thyroid cancer generally use a dose between 150 and 200 μg, with corresponding personal variations. Hypothyroid adults generally use a dose between 50–150 μg depending on the clinical picture (total or partial thyroid ablation, other treatments or pathologies, etc.). Women increase their dosage slightly during pregnancy depending on the state of health. Congenital hypothyroidism requires an age/weight-dependent dosage; the initial dose used in our center is 10–15 μg/kg/day.

**Experimental design**

TSH and free T4 (fT4) were measured with an ultrasensitive method by chemiluminescence (CL) in an automatic analyzer (ACS 180 Plus, Ciba-Corning, Medfield, MA). The normal range established by the laboratory is from 0.2–5.5 μU/mL for TSH and from 9.5–20 pmol/mL for fT4. To check the validity of the technique, the TSH and fT4 determinations were repeated for 65 randomly chosen samples from all those subjects who had had high TSH levels. The second measurement of the TSH hormone was made by immunoradiometric assay (IRMA; Incstar Corp., Stillwater, MN), a technique used until 1995, with a normal range ranging from 0.05–5.0 μU/mL. The fT4 measurement was repeated by RIA, for which the normal levels are 10–20 pmol.

**Tablet assay**

Levothroid tablets from Malaga and Madrid belonging to lot J were analyzed together with other preparations and dosages from diverse origins. In this report, however, we concentrate on the preparation that led to problems in thousands of patients in Spain. The tablets were weighed, dissolved individually in 1 mL concentrated ammonia (25%), and vortexed for 1–2 min, after which 2 mL distilled water [high performance liquid chromatography (HPLC) quality] were added, and the mixture was again vortexed for 2–3 min. As assessed by the addition of tracer amounts of [125I]T4, less than 0.5% of the radioactivity remained in the pellet after centrifugation for 10 min at 2000 rpm at room temperature. The supernatant was separated and used for determination of the T4 content by HPLC and specific RIA or of the iodine content. HPLC. A volume of supernatant containing 2 and 3 μg T4, as calculated from the theoretical T4 content of the tablet, was injected into a C8 column (5 μm, 50 × 2.1 mm) and eluted with 35% methanol-47% 0.015 mol/L ammonium acetate, pH 6.0, at a flow of 0.6 mL/min, using Kontron 325 System equipment and a 332 detector (Kontron Instruments, Milan, Italy). Absorbance was measured at 320 nm. The same procedure was followed with T4 standards (T4 sodium salt from Sigma Chemical Co., St. Louis, MO) ranging from 0.05–5.0 μg, and the results from three different curves were introduced into the database. The T4 content of the sample introduced into the HPLC column was quantified automatically, using the area of the T4 peak. From this value, the initial T4 content of the tablet was calculated.

RIA. One hundred-microliter aliquots of the supernatant were diluted stepwise 10,000-fold using the RIA buffer (0.04 mol/L phosphate buffer, pH 8, containing 0.2% BSA and 0.6 mmol thimerosal; Sigma Chemical Corp., St. Louis, MO) twice to 10 mL. Twenty- and 40-μL duplicates were submitted to a highly sensitive and specific T4 RIA, previously described (12), and the T4 content was quantified against a T4 standard curve run in the same assay.

**I content.** Twenty- and 50-μL aliquots of supernatant were digested with chloric acid, and the iodine content was determined by the Sandell-Kolthoff ceric-arsenite reaction, as described by Benotti and Benotti (13); the iodine content was quantified from iodate standards run in the same assay. The T4 equivalents were calculated assuming all of the iodine to be in the form of T4, using a multiplication factor of 1.53.

Two tablets were assayed from each parcel, each in duplicate, using the three methods, and the entire procedure was repeated on three different occasions. The mean values (±SD) are reported.

**Statistical analysis**

Statistical comparison of the mean levels in each group was made using Student’s t test, and the studies were correlated using Pearson’s test.

**Results**

The first modifications detected corresponded to adult hypothyroidism in which gradually higher serum TSH levels were seen. The significant increase in TSH in CH began to be noticed during the first half of 1995. This group of patients was the most closely followed due to the high number of controls to which they were submitted (11). Retrospective study showed the following percentages in the appearance of high cases: first half: 1994, 8.77%; 1995, 26.31%; and second half: 1994, 7.01%; 1995, 57.89%.

Given the increase in the number of patients receiving treatment with high levels of TSH, the ultrasensitive CL technique was reevaluated, comparing it with another immunoassay method (IRMA), with which the Molecular Endocrinology Laboratory also had experience. The means of the levels obtained by each technique for both TSH and fT4 showed no significant differences when Student’s t test was applied for the nonpaired samples, although the TSH data obtained by CL were superior to those obtained by IRMA when these were paired (P < 0.01); there was a good correlation between both methods (r = 0.96). A multicenter external control was also made with four laboratories that confirmed the validity of the results.

The control group was formed by a homogeneous population from a village near Malaga. The mean TSH level was 1.66 ± 0.89 μU/mL (range, 0.32–4.2 μU/mL), and the mean fT4 level was 15.58 ± 1.68 pmol/mL (range, 11.3–19.0 pmol/mL).

Of the 267 AH, 167 (65.5%) had TSH levels higher than 6 μU/mL, 87 (32.5%) had TSH levels between 0.2–5.5 μU/mL, and 13 (4.8%) had suppressed TSH, with levels below 0.2 μU/mL.

The 84 HC were distributed as follows: 42 patients (50%) had TSH levels above 6 μU/mL, 38 (45%) had TSH levels between 0.2–5.5 μU/mL, and another 40 (37%) had TSH levels in the normal range. These 2 groups with inadequate TSH levels comprised 70% of all TC receiving treatment (n = 108). There were 32 (29%) TC with suppressed TSH.

Of the eight PH, seven (78.5%) had TSH levels above 6 μU/mL at the last control, and one (12.5%) had a normal TSH level (Table 1 and Fig. 1). The amount of in vitro levothyroxine was measured by RIA, HPLC, and its iodine content after dissolving the tablets in an ammonia solution (Table 2). The lowest levels of levothyroxine found in the 50-μg Levothroid tablets were determined by RIA, with a mean level of...
TABLE 1. Etiological distribution of patients in order of TSH levels

<table>
<thead>
<tr>
<th>Patients</th>
<th>TSH &gt;6 (µU/mL)</th>
<th>TSH = 0.2–5 (µU/mL)</th>
<th>TSH &lt;0.2 (µU/mL)</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult hypothyroidism</td>
<td>167 (62.5)</td>
<td>87 (32.5)</td>
<td>13 (4.8)</td>
<td>267</td>
</tr>
<tr>
<td>Thyroid cancer</td>
<td>36 (33)</td>
<td>40 (37)</td>
<td>32 (29)</td>
<td>108</td>
</tr>
<tr>
<td>Congenital hypothyroidism</td>
<td>42 (50)</td>
<td>38 (45)</td>
<td>4 (5)</td>
<td>84</td>
</tr>
<tr>
<td>Totals</td>
<td>244 (53)</td>
<td>166 (36)</td>
<td>49 (11)</td>
<td>459</td>
</tr>
</tbody>
</table>

Percentages are given in parentheses.

32.3 µg, resulting in a 35.3% loss of activity. The mean levothyroxine level in these same tablets determined by HPLC was 39.3 µg, a 21.3% loss of activity. There was no significant loss of iodine in these tablets, with a mean experimental value of 48 µg. The correlations among the three techniques were: iodine/HPLC, \( r = 0.900 \); RIA/HPLC, \( r = 0.870 \); and RIA/iodine, \( r = 0.969 \). The samples of 100 µg analyzed presented even higher amounts than indicated by the commercial laboratory.

**Discussion**

Control of the treatment of hypothyroidism has long been controversial. Apart from the difficulty in establishing a permanent euthyroid state resulting from different individual responses (1–3, 7, 14–16), divergence in the bioequivalence of different commercial preparations and differences between the amount of active ingredient indicated by the manufacturer and the actual dose contained in the tablets have been reported (3, 6–8). The aim of substitution therapy with \( fT_4 \) is to maintain normal TSH levels, measured with third generation assays, without the necessity of establishing a euthyroid state with the TRH test (1, 2, 10).

The development of extremely sensitive tests for measuring serum TSH has resulted in the attending physicians placing more trust in biochemical results than in clinical appreciations when these are conflicting (16). This trust, however, can be put to the test when a significant number of results of biochemical evaluation of the treatment of hypothyroidism present over a short period of time modifications in the levels of TSH that had previously been well controlled.

In 1995, when gradual increases in TSH figures began to be found in patients treated with levothyroxine (17, 18), it was initially thought to be a problem related to the hormone tests in the Molecular Endocrinology Laboratory. Apart from both internal and external controls, the validity of the technique was checked by reevaluating a series of randomly chosen samples from among those with high TSH levels made with CL using an IRMA technique. The correlation between the methods was significant; there were no differences between the means, although the TSH levels obtained by CL were higher than those obtained by IRMA when they were paired and contrasted. The nonmodification of the remaining routine values of the thyroid studies, the good grouping in the control population, and the results of functional studies also support the validity of the results.

The correlation existing between the two assay techniques and the evolution of \( fT_4 \) and TSH levels over time (Fig. 2) suggest that the lineal drop in \( fT_4 \) was due to a reduction in the dose (3) and that the exponential increase in TSH may be due to the normal negative feedback response of TSH to the decrease in \( T_4 \) (16, 19), which continues to be operative in patients receiving exogenous \( T_4 \) (20).

The reasons for the increased TSH levels in hypothyroid patients undergoing treatment may be due to normal fluctuations, which are very frequent when exogenous \( T_4 \) is being administered (1); lack of collaboration by the patient (2); loss of activity of the preparation over time (6); a modification in intestinal absorption on changing lots (3); variations in the amount of active ingredient (7); interference of
the preparation by ingested fiber (4); or a different bioequivalence with the same amount of preparation (21).

Data from the analysis of pharmaceutical preparations agree with the clinical findings. The greater loss of levothyroxine in the tablets from lot J, which has already been withdrawn by the commercial laboratory, was found in the quantitative by RIA, which in a recent work showed normal values (22). Quantification by HPLC showed less deficit, whereas the amount of iodine varied very slightly compared to the theoretical content. This might suggest a loss of biological activity due to deterioration of the tablets either during or after the elaboration process. Both the company itself and the Spanish Ministry of Health carried out studies on the lots involved, agreeing that there were significant differences in the speed of dissolution in vitro between the original product from the U.S. and the recently imported product from France (23), concluding that the hypothesis of bioequivalence between the tablets manufactured from the two types of raw material was confirmed in vitro, and that it could explain the clinical responses detected due to a lower bioequivalence of the tablets made from nonmircronized levothyroxine, with an in vivo study of bioavailability being unnecessary.

The clinical consequences of the data presented in this study cannot be generalized. The CH require treatment according to age and period of development (11, 24, 25), and they should be reevaluated. Thirteen AH have suppressed TSH, which implies a risk of atrial fibrillation in older persons (26). Those pregnant women who were taking Levothroid require special treatment. The increase in TSH that appears during pregnancy in hypothyroidism (2) should be borne in mind to maintain the required euthyroid state during this period when changing the preparation (10). Likewise, those patients who have had their TSH normalized with an increase in the dose of the old preparation run a severe risk of an overdose if they maintain the same treatment with the new tablets. Finally, the TC should be urgently reviewed given the risks that high levels of TSH pose in these patients (2).

An additional problem is the extra cost involved in reevaluating all those hypothyroid patients treated with this drug. The change in preparation is causing problems for both patients and physicians, as the number of tests has increased by 100%. For example, between September and December 1996, 40% of the patients with thyroid cancer did not have suppressed TSH levels, and 29.4% of the congenital hypothyroid patients and 23.7% of the adult hypothyroid patients had elevated TSH levels several months after the changeover. We suppose that the final balance in problems of this type lies somewhere in between the economic interests of the company, the competence of the physicians, and the demands of the patients.

References