THYROID FUNCTION DURING THE FIRST YEAR OF RECOMBINANT HUMAN GROWTH HORMONE THERAPY IN SHORT STATURE CHILDREN

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THYROID FUNCTION DURING THE FIRST YEAR OF RECOMBINANT HUMAN GROWTH HORMONE THERAPY IN SHORT STATURE CHILDREN (abstract) Introduction: The relationship between rhGH treatment and thyroid function has been the subject of numerous studies. Some say that rhGH treatment unmasks central hypothyroidism, other say that rhGH induces subclinical primary hypothyroidism. Aim: To assess the changes in thyroid function in short stature children in the first year of treatment with rhGH and the impact on growth velocity. Material and methods: We evaluated 37 patients treated with rhGH, 5 were excluded because developed side effects during treatment. We measured height, height velocity, and height standard deviation gain during treatment and thyroid function during the first year of treatment. Results: We observed a slight increase in the TSH level and no significant change in the f T<sub>4</sub> level after the first 3-6 months of treatment in all the groups; in GH deficiency (GHD) patients, we observed a statistically significant decrease of the f T<sub>4</sub> level after the first 3-6 months, without a significant increase of the TSH level. After the first year, thyroid function returned to baseline. There were no differences between height velocities in all the groups, except from the GHD patients. Conclusions: The slight increase in the TSH level and the decrease of f T<sub>4</sub> level might unmask a transient subclinical primary hypothyroidism but these changes do not influence the growth velocity in first year of rhGH treatment. Keywords: RECOMBINANT HUMAN GROWTH HORMONE (rhGH), THYROID FUNCTION, TSH, FT<sub>4</sub>

Recombinant human growth hormone (rhGH) has been approved for the treatment of growth deficiency (GHD) in both children and adults since 1986 and also since then, the indications have been extended to other non-GH deficient diseases in children, such as idiopathic short stature, Turner syndrome, small for gestational age, Prader–Willi syndrome. rhGH can be given as a replacement dose (for GHD) or pharmacologic (for most other conditions).

In children with GHD, rhGH treatment is given in doses that reproduce the daily normal secretion of growth hormone. In pharmacological dose, rhGH stimulates growth-promoting pathways affected by the diseases or not functioning properly (1). In these cases rhGH is used in higher doses in order to overcome the relative GH or IGF-1 insensitivity. The response to treatment depends
on the sensitivity to GH, the dosage and the age of initiation of the treatment (2).

The relationship between rhGH and hypothalamus-pituitary-thyroid axis has been the subject of several studies with divergent results. Researchers showed that rhGH treatment induces a fall in serum total and free T₄ concentration, an increase in serum T₃ concentration, independent of the TSH action, which decreases or remains unchanged. Several explanations have been issued for these effects but most of them are contradictory (3-8).

The aim of our study was to evaluate the changes induced by the rhGH treatment on the thyroid function and the impact on velocity in short stature children in the first year of treatment with rhGH.

**MATERIAL AND METHODS**

We evaluated children treated with rhGH between 2009 and 2012, in our Department of Endocrinology. Patients were included in our study based on the inclusion criteria for rhGH treatment of the current national recommendations in the program of therapy of short stature children (9). Parental informed consent was obtained from all the participants in our study.

The retrospective analysis included 37 patients. We divided our patients in 4 subgroups: growth hormone deficient (GHD) 17 patients, Turner syndrome 4 patients, small for gestational age (SGA) 5 patients and idiopathic short stature (ISS) 6 patients (tab. I).

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>GHD</th>
<th>Turner Sdr.</th>
<th>SGA</th>
<th>ISS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>17</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Female/Male</td>
<td>7/10</td>
<td>4</td>
<td>3/2</td>
<td>2/4</td>
<td></td>
</tr>
<tr>
<td>Age at GH start, years</td>
<td>10.46±3.5</td>
<td>10.25±3.59</td>
<td>9±3.39</td>
<td>9.33±1.42</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Target height, cm</td>
<td>171±9.8</td>
<td>165.5±2.04</td>
<td>168.4±8.99</td>
<td>170.58±4.85</td>
<td>&gt;0.9</td>
</tr>
<tr>
<td>Bone age at GH start</td>
<td>6.72±3.33</td>
<td>10.653±2.08</td>
<td>5.906±4.82</td>
<td>4.045±1.66</td>
<td>0.0868</td>
</tr>
<tr>
<td>rhGH dose, µg/day</td>
<td>0.70±0.26</td>
<td>1.23±0.57</td>
<td>0.6±0.25</td>
<td>0.72±0.101</td>
<td></td>
</tr>
<tr>
<td>I-st year velocity, cm/y</td>
<td>8.59±2.19</td>
<td>7.625±0.47</td>
<td>7.88±1.08</td>
<td>8.26±0.737</td>
<td>0.129</td>
</tr>
<tr>
<td>Height SD gain</td>
<td>0.94±0.61</td>
<td>0.775±0.38</td>
<td>1.196±0.46</td>
<td>1.192±0.29</td>
<td>0.5981</td>
</tr>
</tbody>
</table>

| Maxim GH, ng/ml          | Clonidine | 6.76±2.96 | 16.75±2.93 |       |
|                          | Insulin   | 4.93±3.38 | 6.98±5.6  |       |
| TSH, mUI/l (0.38-4.31)   | Before    | 2.46±0.40 | 4.66±1.76 | 2.43±0.77 | 2.026±0.6 | 0.34 |
|                          | 3-6 mo    | 3.21±0.48 | 5.35±1.34* | 3.31±0.55 | 1.65±0.39* | *0.039 |
|                          | 1 year    | 2.28±0.39 | 3.42±1.39 | 3.42±0.17 | 2.32±0.57 | 0.5159 |
| fT₄, ng/dl (0.82-1.63)   | Before    | 1.39±0.084 | 1.31±0.14 | 1.20±0.04 | 1.28±0.046 | 0.5451 |
|                          | 3-6 mo    | 1.08±0.07* | 1.22±0.088 | 1.40±0.03* | 1.29±0.05* | *0.0454 |
|                          | 1 year    | 1.16±0.075 | 1.2±0.05 | 1.26±0.04 | 1.08±0.107 | 0.4274 |

*groups compared to one another, with statistical significant difference (p<0.05)

Patient height was obtained by direct measurement on a wall mounted stadiometer and standard deviation was assessed using Prader 1989 growth charts. Target height was calculated using the formula: (father’s height+mother’s height ± 13 cm)/2. Bone age was calculated according to Greulich-Pyle’s 1959 standards (10) or Tanner Whitehouse 20 system. All the stimulation tests and blood samples were...
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performed during the morning hours.

Diagnosis of GHD was based on height below - 2.5 standard deviations (SD), bone age delayed with more than 2 years, low or normal IGF-1 for age and sex and at least 1 negative stimulation test for growth hormone. We performed clonidine stimulation test with 0.15 mg/m² or insulin tolerance test with 0.15 IU/kg. GHD was established if GH peak was less than 10 ng/ml. Severe GHD was assessed as peak GH less than 3 ng/ml.

Diagnosis of SGA was based on birth weight less than -2.5 SD or length less than – 2 SD compared to normal values for gestational age, height below – 2.5 SD at the age of 4.

ISS was diagnosed in the presence of a height less than - 3 SD, with normal nutritional status, no history of chronic diseases and after having excluded any other cause of growth failure.

Diagnosis of Turner syndrome was made on the clinical phenotype and confirmation by karyotyping of peripheral blood leukocytes. Diagnosis for Noonan syndrome was made clinically and Prader-Willi syndrome was diagnosed based on methylation analysis.

We excluded children less than 3 years of age and we did not start treatment until we excluded other possible secondary causes for growth failure like decompensated cardiac abnormalities, decompensated renal failure, malabsorption, congenital hypothyroidism, Cushing syndrome, decompensated diabetes mellitus, skeletal deformations, vitamin D deficiency, pituitary tumors, active malignant tumors or genetic disorders other than Turner syndrome, Noonan syndrome, Prader-Willi syndrome or Russel Silver (approved in our country for rhGH therapy).

Clinical aspects. The patients have been regularly followed up at our clinic. Before starting rhGH treatment; we assessed all the antropometric parameters, like birth height and weight, actual length and weight, target height, bone age and signs of puberty. We also obtained magnetic resonance images of the sellar region in order to exclude possible intracranial tumors. MRI showed partial empty sella in one girl with Turner syndrome, complete empty sella in one SGA boy, agenesis of corpus callosum in a GHD girl and pituitary hypoplasia in 2 children with GHD.

During each visit at 3, 6, 9 and 12 months after initiation of rhGH therapy, patients were closely checked for body height, weight, bone age, signs of pubertal development, complete blood count, fasting glucose levels and thyroid function – TSH, fT₄ and thyroid antibodies in case of hypothyroidism.

Treatment. Thyroid function was normal in most of the children (24 cases) but in the remaining 8 cases, 3 in GHD group, 3 in Turner group, one in SGA group and one in ISS group, levothyroxine was administered for primary hypothyroidism in order to maintain TSH and fT₄ values within the normal range and the dose was adjusted at each visit during follow-up. Three children had positive thyroid anti peroxidase antibodies.

All the patients except one girl were prepubertal at the time of diagnosis and no priming was necessary. The girl had Turner syndrome and conjugated estrogen therapy was started one year before initiation of rhGH therapy. Bone age was delayed in all groups with the exception of Turner patients with a bone age similar to the chronological one.

The dose of rhGH was adjusted in order to maintain an IGF-1 level within the normal range for sex and age.

Plasma TSH and fT₄ concentrations
were measured using an electrochemiluminescence immunoassay „ECLIA”. Growth hormone concentrations were measured by IMMULITE® 2000 Growth Hormone. IGF-1 was assessed by an automated chemiluminescent assay system (IMMULITE®, Diagnostic Products Corp., Los Angeles, CA, USA) and the normal range was corrected for age.

Statistical analysis was performed in GraphPad prism 6, Demo Version. Numerical data are expressed as mean ± SD. We used t-test for paired data, Wilcoxon’s test for non-parametric data with non-Gaussian distribution. When we analyzed the differences between more than three groups, we used ANOVA test. The level of statistical significance was at p < 0.05.

RESULTS

During follow up, we excluded 5 patients: one girl developed type 1 diabetes mellitus 8 months after rhGH treatment, one boy developed restrictive cardiomyopathy after 4 years of treatment, one boy presented high glucose levels 1 year after treatment, one girl with Prader-Willi syndrome presented aggravated obstructive sleep apnea 3 months after starting rhGH treatment and one boy was lost during follow-up. No other serious side effects were reported, except for a suspicion of epiphysiolysis in 14 years old, unconfirmed by the orthopedics.

We observed a slight increase without statistical significance in the TSH level and no significant change in the fT4 level after 3, 6, 9 or 12 months of treatment in all the groups (tab. I, fig. 1A, B). The increase of the TSH level was highest after the first 3-6 months of treatment and during the first year it returned to pretreatment levels. The small changes of fT4 level also stabilized after the first year of rhGH treatment (fig. 1C).

![Fig. 1. Thyroid function evolution during the first year of rhGH treatment.](image-url)
Thyroid function during the first year of recombinant human growth hormone therapy in short stature children

We compared TSH values between the 4 groups at the same time during monitoring. We observed a statistically significant difference of TSH values at 3-6 months only between Turner patients and ISS patients (tab. I).

Despite these changes, none of the patients from the euthyroid group needed levothyroxine supplementation as compared to four of the patients previously treated with levothyroxine who needed a small increase of the dose with an average of 25µg/day in order to maintain TSH values within the normal range.

In a subgroup analysis for GHD patients, changes of the thyroid function were more evident, with a significant fall in serum fT\(_4\) levels after 3 and 6 months of rhGH therapy (fig. 2B). fT\(_4\) was towards the lower limit but always within the normal range and we decided not to start levothyroxine substitution therapy. We did not find a significantly increase in the TSH level after the first months of treatment (fig. 2A) as we would have expected based on the fall of fT\(_4\) levels.

![Fig. 2. Thyroid function in the GHD patients during the first year of rhGH treatment.](image)

There were no statistical differences between height velocities and height standard deviation gain in the first year of rhGH treatment in all groups (tab. 1, fig. 3).

![Fig. 3. Height velocity during the first year of rhGH treatment.](image)
The greatest height velocity was observed in the GHD group while height standard deviation gain was the most important in SGA group. The lowest gain was seen in Turner group. We did not notice any differences regarding velocity and height standard deviation gain between the euthyroid patients and those with previous levothyroxine substitution before rhGH therapy. In the GHD subgroup we observed a statistical difference in the height velocity between those who had a maximum GH during clonidine stimulation test less than 7 ng/ml versus those with maximum GH more than 7 ng/ml (fig. 3B).

**DISCUSSION**

This study demonstrates that during rhGH therapy for short stature children, thyroid function is influenced by a shift of fT4 levels toward the lower limit and slightly elevated levels of TSH in all euthyroid children. The same changes were also seen in the patients with previous primary hypothyroidism but adequately substituted with levothyroxine.

Based on the results of our study, we issued several hypotheses. One of them is that rhGH treatment induces an increase in the activity of deiodinase type I and III which leads to increased peripheral conversion of T4 to T3. The increased levels of T3 might lead to decreased secretion of TSH through negative feedback. Also, the decreased levels of fT4 induce through negative feedback an increased secretion of TSH. Together, these might explain why TSH level remains relatively low as compared to the decreased level of fT4, such as seen in central hypothyroidism. Of great interest would be to determine the effect of rhGH treatment on the TRH gene expression in the hypophysiotropic neurons and the activity of type 2 deiodinase (D II), the primary thyroid hormone activating enzyme in the mediobasal hypothalamus and also in peripheral tissues. Another hypothesis of ours is that rhGH induces inhibition of TRH gene expression by increasing the local thyroid hormone activation in the hypothalamus and leading to central subclinical hypothyroidism. In addition, we hypothesize that an increase of DII activity in peripheral tissues may also contribute to the altered peripheral T3/T4 ratio.

Previous studies have shown that rhGH therapy might unmask a central hypothyroidism in the face of low fT4 with inadequately elevated TSH levels (5). Others contest this explanation by showing that fT4 changes induced by rhGH therapy are independent of TSH secretion and are caused by the increased peripheral conversion of T4 to T3 which will determine a blunt TSH secretion via negative feedback (6). Because of these effects on thyroid hormones, Portes et al suggest that serum T3 levels have no use in the diagnosis of central hypothyroidism but they would be useful to indicate excessive thyroxine supplementation (6).

Since we could not measure the T3 serum concentration to justify the physiological response of TSH secretion, we interpret the changes seen in our patients as a transient subclinical central hypothyroidism which resumed after the first year of rhGH therapy. A same hypothesis issued Seminara et al (7).

Despite these changes in the thyroid function, we did not start levothyroxine substitution in our previous euthyroid patients because none of the values of fT4 were below the normal limit and none of our patients developed signs or symptoms suspicious of hypothyroidism. We in-
creased the dose of L- T₄ in only four patients with previous primary hypothyroidism because of increased TSH levels above the normal limit, especially in the group of patients with Turner syndrome. As it has been shown, children with Turner syndrome are predisposed to thyroid disorders, especially hypothyroidism induced by thyroid autoimmunity, leading to increased levels of TSH and low or normal levels of fT₄ (11). This mechanism also explains the significant statistical difference we observed when comparing the TSH value at 3-6 months between Turner patients and ISS patients.

When comparing fT₄ levels we observed a statistical difference between the lower fT₄ values in GHD patients compared to fT₄ levels in SGA patients. We explain this difference as a direct effect of rhGH supplementation in GHD patients and increased peripheral conversion of fT₄ to T₃, as compared to SGA patients.

We assume that the transient changes in TSH and fT₄ are a consequence of a new equilibrium established once rhGH therapy has been started, especially in those with growth hormone deficiency. The transient changes of thyroid function seen in our study were also reported by other researchers but the mechanisms remain unclear. Besides the increased peripheral conversion of fT₄ to T₃ through stimulation of deiodinase type I and III, and decreased conversion of T₄ to r T₃ (8), some say that the extracellular water increase might determine these shifts in thyroid hormone levels (12). Another possible explanation for the lack of significant changes in TSH level is the increased somatostatinergic tone through somatostatin, the natural inhibitor of TSH during rhGH therapy (13).

Despite these changes, we did not notice any negative impact on growth velocity and height standard deviation gain in either of the studied groups. The positive growth response might be explained by the increased levels of serum T₃ actively acting in periphery. All patients had velocities expected for the treatment schedule. There were no changes in velocity when comparing euthyroid patients to those with l-thyroxine substitution before the onset of rhGH therapy which further supports our hypothesis that these changes in the thyroid function are transient and there is no need for l-thyroxine substitution unless there are clear changes suggestive for hypothyroidism, especially in those patients previously known with thyroid diseases.

**CONCLUSIONS**

During the first year of rhGH treatment, there are changes in the thyroid function, with a slight increase in the TSH level and a decrease of fT₄ level. We suppose that these changes unmask a subclinical primary hypothyroidism but do not influence the growth velocity. There should be a careful decision for supplementation with l-thyroxine in previous euthyroid children, since the changes in thyroid function are transient and return to baseline values within one year of continuous treatment with rhGH. Further studies are necessary in order to establish the effects of rhGH treatment at the hypothalamic and pituitary level and the impact on thyroid hormone metabolism.

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**Declaration of interest**

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific paper.
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