UNIVERSITY OF MEDICINE AND PHARMACY
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ABSTRACT

DOCTORAL THESIS

ENDOCRINO-METABOLIC SEARCHES IN THE SHORT STATURE. DIFFERENTIAL THERAPEUTIC ATTITUDES

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THE KEY WORDS: HEIGHT HYPOTROPHY, GH-DEFICIENCY, IDIOPATHIC SHORT STATURE, GHR gene

THE KNOWING LEVEL

CHAPTER I. THE NORMAL HEIGHT

We present some information regarding the growth genetics: the developing of the pituitary and its impact on the hormonal deficiencies; transcription factors with clinical importance. As well, the most recent information are commented from the specialized literature regarding the components of the axis GHRH-GH.

CHAPTER II. THE ETIOPATHOGENESIS AND THE DIAGNOSE OF THE HEIGHT HYPOTROPHY

For a medication to be efficient in the growing disorder, it must be recommended on the base of a diagnose of growing delay. This is established after some investigations that are meant to discover the modifying mechanisms of the growing disorder. For this purpose, the modifying classifications of the huge growing disorders are welcome.

In this chapter we insist on the etiology and the diagnose of the height hypotrophy due to the endocrine disorders.
CHAPTER III. DIFFERENTIAL THERAPEUTIC ATTITUDES
Initially, the rhGH treatment was used on the children with growth hormone deficiency (GHD). Then, these indications were extended to other medical problems, that begin with short stature and they are not associated with GH deficiency.

The patients with GHD can be treated with genetic recombined GH as soon as possible for establishing the diagnose. The main objective of the therapy is represented by normalizing the height during the childhood and the obtaining of a normal height as an adult.

The treatment with human recombinat IGH-I –rhIGF-I- was approved in The United States of America and Europe in the treatment of the patients with severe primary IGH-I deficiency or for the patients with deletions of the GH1 gene, which have developed anti-GH antibodies. RhIGH-I could be used in the future on the patients with partial resistance at GH or on those with idiopathic short stature, but until present there are not enough information in order to make such recommendations.

OWN CONTRIBUTIONS

The purpose of the work is to study the metabolic and the hormonal changes of the children with growing disorder and to appreciate the results of the treatment for promoting the growingin the case of hypotrophy stature, this due to the somatotrope deficiency or not.

The specific objectives we proposed to realize in this study are: 1) the study of the clinical and anthropometrical criteria of the patients with hypotrophy stature, these due or not to the GH deficiency; 2) the earliest diagnose of GH deficiency; 3) the metabolic and hormonal evaluation of the studied patients; 4) the appreciation of the results of the growth promoting treatment with genetic recombinant growing hormone at the patients with GH deficiency; 5) finding the possible flaws of the GH (GHR) receptacle gene at the children with idiopathic short stature (ISS); 6) the evaluation of the results of the treatment with rhGH at the children with idiopathic short stature.

Chapter II. METHODS
The study group is formed by 92 children with hypotrophy stature, who came to the Clinic of Endocrinology and to the Pediatric Clinic of the
Emergency Count Hospital from Craiova, between 2005-2010: 59 patients with growth hormone deficiency, 24 children with idiopathic short stature (ISS), 9 patients with Turner female syndrome.

The selection of the patients was made on clinical criteria and on laboratory investigations: usual, hormonal, cytogenetic, imaging, molecular, all of these in order to find the possible flaws of the growth hormone receptacle gene.

THE GENERAL ANALISE OF THE STUDIED PATIENTS

- Pituitary deficit of GH - 59 patients, aged between 3-17 years old, out of them 17 are girls (29%) and 42 boys (71%), with a ratio M/W = 2.47. The patients with ages between 3-13 are in number of 47 (80%), and those aged between 14-17 are 12 (20%).

- Idiopathic short stature (ISS) - 24 patients, aged between 4-16 years, 11 boys and 13 girls.

- Female Turner syndrome - 9 patients, aged between 3-19 years (3-11 years old 2 patients, 12-19 years old 7 patients).

THE WORKING PROTOCOL

For including the children with GH deficiency into the study group the following parameters were taken into account:

- The delay in growing with 2 or more standard deviations (SD) besides the media for age and sex.
- A slow growing speed during the last year.
- The normal height of the parents.
- Suggestive clinical characteristics for somatotrope deficiency.
- Morphotip harmonic (the pituitary dwarfism is harmonic).
- The clinic history for excluding a psycho-social dwarfism.
- The delayed bone age < than the chronological age (a delay of at least 2 years).
- At two stimulation tests the value of GH <10 mUI/I.
- The basic value of GH is low (or normal, but correlated with the post –stimulation values <10m UI/I).

The general characteristics of 59 children with GH deficiency are resumed in the table no. 1.
Table no.1- The general characteristics of the studied group

<table>
<thead>
<tr>
<th>Parameter</th>
<th>n</th>
<th>Media</th>
<th>Dev.std.</th>
<th>C.V. (% Ds/M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>59</td>
<td>10.65</td>
<td>3.35</td>
<td>31.42</td>
</tr>
<tr>
<td>BA</td>
<td>59</td>
<td>7.69</td>
<td>3.15</td>
<td>40.94</td>
</tr>
<tr>
<td>Delay BA</td>
<td>59</td>
<td>2.96</td>
<td>0.66</td>
<td>22.26</td>
</tr>
<tr>
<td>The age of the height</td>
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<td>2.78</td>
<td>37.21</td>
</tr>
<tr>
<td>The score Z</td>
<td>59</td>
<td>-2.99</td>
<td>0.83</td>
<td>-27.61</td>
</tr>
<tr>
<td>IMC</td>
<td>59</td>
<td>16.19</td>
<td>2.07</td>
<td>12.76</td>
</tr>
<tr>
<td>MPH</td>
<td>59</td>
<td>169.98</td>
<td>6.41</td>
<td>3.77</td>
</tr>
</tbody>
</table>

The Turner syndrome with female phenotype is classical defined through the following elements: marked and disharmonic hypotrophy stature (more than 2.5 SD under the average for the age); multiple somatosensory visceral anomalies: skeletal, cardiac, renal, bone, etc; severe ovarian failure through anovarie; delayed puberty with plasma and urine growing of the levels of the gonadotropic hormones; specific modified cariotype, with a single gonosome X-45,XO.

At the 9 patients with Turner syndrome from our group, we noticed hypotrophy stature without exception, with variations between 88-141 cm.

For assess the growth delay besides the average for age and sex, we calculated the Z score of the height, which varied between -2,20 SD and -5,39 SD, with an average value of -4,19 SD. In the 2 patients with ages between 3-11 years the growth delay was less severe (-2,73 SD and -2,20 SD); for the patients aged over 12 years, the Z score had values between -3,95SD and -5,39 SD. The height age of those 9 patients had an average of 8.05 years, and for IMC the average value obtained was 22,95 kg/m2.

**Chapter III. RESULTS**

**METABOLIC INVESTIGATIONS**

The Carbohydrate metabolism. Finding the values of the a jeune glucose of the 59 patients with GH deficiency included in our group of study showed the following: normal values (77-110 mg/dl) at 30 patients (50.85%), 22 boys and 8 girls; low values (60-77 mg/dl) at 29 patients (49.15%), 19 boys and 10 girls.

At the 9 patients with Turner syndrome the values of the a jeune glucose were normal.

The Lipid Metabolism. The evaluation of the serum levels of the total cholesterol, of the lipids and of the triglyceride showed the following results at the patients with GH deficiency: total serum cholesterol was determined at all the 59 children from our group of study, out of these 7 cases (11.86 %)
indicated high values, and the others left -52 in number (88.14%) indicated normal values; the level of the serum triglycerides was found at 23 patients, out of these 21 had normal values and only 2 had high values; lipids was determined at only 14 patients, all of them indicating normal values.

Determination of the values of the total cholesterol at the 9 patients with Turner syndrome showed the following: normal values at 6 cases; high values at 3 cases.

HORMONAL INVESTIGATIONS
PITUITARY DWARFISM
THE DETERMINATION OF THE GROWTH HORMONE (GH). The basic values of the growth hormone at 30 patients were low: 0.012-0.47 ng/ml (normal: 0.5-7 ng/ml), and at 29 children the values situated at the lower limit of the normality: 0.5-1.23 ng/ml.

Tests for stimulating GH secretion:
- The test of the insulin-induced hypoglycemia (TTI) was made at 51 (86.44%) children from our group of study, the maximum values of the post-stimulation GH did not exceed the level of 10 ng/ml at any of the patients.
- The test at arginine was made on 24 patients and, as well, the maximum level of the post-stimulation GH did not exceed 10 ng/ml.
- The test at clonidine was made on 11 patients.

The determination of IGF1. The value of IGF1 was determined at all the 59 children from our study. At 26 (44.07%) patients we found low values, and at 33 (55.93%) the values were between normal limits.

The determination of TSH, FT4, FT3, ATPO. TSH, dosed at all the patients of the group, showed normal values at 47 (79.66) children and high values at 12 (20.34) children. At the 12 children with high TSH, FT4 and FT3 had normal values or at the low limit of the normal. We mention that ATPO, determined at all the patients, had a high value at only 1 patient, but TSH,FT4 and FT3 were between normal limits.

The determination of PRL and of the gonadotropin hormones. At 12 children, age between 14-17 years, gonadotropin hormones and prolactin were determined. PRL had normal values at 11 patients; it was high at patient B.R., aged 14.1, but performing MRI skull indicated a normal aspect of the pituitary gland, while FSH and LH were between normal limits.

FSH and LH had normal values at 11 patients. The patient V.C., aged 14 years, had low values, with nontriggered puberty (P1G1). This child had also low values at the plasma cortisol and 17-urinay-cetosteroids, normal PRL, high TSH. The radiography of Turkey saddle and the RM skull exam had normal aspect.
THE TURNER SYNDROME

The values of the ovarian hormones (E-estradiol and P-plasma progesterone) were low, while those of the gonadotrophic hormones had values that exceed the high level of normality. The values of the ovarian hormones and those of the gonadotropes raised our interest especially at 7 patients with Turner syndrome, aged 12-19 years.

The growth hormone in the case of 2 (12.3 years old and 14.9 years old) from the 9 patients with Turner syndrome had low basic value, while IGF1 showed values between normal limits at 7 patients and low values at the other 2 girls.

Prolactin was as well determined at all the patients with Turner syndrome; in all the cases the obtained values were normal.

TSH determination indicated raised values in the case of 2 patients, the other 7 having normal values.

IMAGING INVESTIGATION

The radiography of Turkey saddle- at 3 of the patients with GH deficiency we noticed a particular aspect of small saddle, “in omega”. At the patients with Turner syndrome we did not notice any modification of the Turkey saddle.

The CT/IRM exam of the skull – three of the patients with GH deficiency had a pituitary gland with small dimensions.

The punch radiography- at the patients with pituitary dwarfism the bone age (BA) varied between 2-13 years, with an average of 7.69 years. The delay of the BA, calculated as a difference between the chronological age and the bone age, was at least 2 years, with an average of 2.96 years. At all the patients with Turner syndrome BA was coordinating with the chronological age, at 4 of them we noticed a short metacarpal IV.

CYTOGENETIC SEARCHES were made in order to confirm the cases of Turner syndrome.

THE EVALUATION OF THE RESULTS OF THE TREATMENT FOR GROWTH PROMOTING WITH rhGH

From the total of 59 children with GH deficiency, 47 followed, for a year, a treatment with genetic recombinant growth hormone. At these patients we took into account:

A. The modification of the anthropometric parameters under therapy.
B. The relations between different parameters and the answer to the treatment, evaluated in number of cm gained in a year of GH administration (the growth speed).
After a year of treatment we noticed at the patients the following issues: the growth speed under treatment (cm/year), calculated by the difference between the height of the patient at the end of the treatment and the his height at the beginning of the treatment; the Z score of the height after a year of treatment (in SD); the difference between the Z score of the height after a year of treatment and the Z score at the beginning of the treatment.

After a year of treatment we noticed at our patients a significant improvement of the growth speed, comparatively with the year prior the beginning of the therapy, as well as an improvement of the short stature with 0,74SD. The growth speed under treatment and the Z score of the height had comparable values to boys and also to girls (9,48cm/year vs. 9,53cm/year; -2,21 SD vs. -2,58SD).

We have looked for correlations between the growth speed during a year of treatment at all the 47 patients and the bone age at the beginning of the therapy. The Pearson r coefficient of correlation between the two parameters had a value of -0.288, which means a significant statistic inverted correlation (p<0.05), but not very strong, I mean the bigger the bone age, the slower the growth speed was.

We also found at our patients an inverted correlation, significantly statistic (p<0.05), between the growth speed under treatment and the delay of the bone age bigger than 3 years, that means the bigger the delay of BA, the slower the growth is.

The Pearson r coefficient of correlation between the growth speed after a year of treatment and the maximum peak of GH at the test of stimulation with arginine is of -0.161, which shows a weak inverted correlation, insignificantly statistic due to the small number of patients tested, that means the higher the peak, the slower the growth. At our group we did not find correlations between the growth speed under treatment and the peak of GH post-stimulated with insulin and clonidine.

MOLeCULAR INVESTIGATIONS

At a small group of 24 children with idiopathic short stature (ISS), we performed some molecular investigations in order to find the possible flaws of the gene of the growth hormone receptor. We made analyses regarding the SSC polymorphism (single-strand conformation) of the gene of the GH receptor.

The 24 children, aged between 4-16 years (11 boys and 13 girls) had the following characteristics:
- had a growth delay bigger than 2.25SD under the average height for age and sex (an average of -3, 24 SD), without presenting systemic, endocrine or nutritional abnormalities, as it is the definition of ISS;
- at some children the GH was between normal limits, while at others GH had a small basic value, but which grew over 10 ng/ml at the insulin-induced hypoglycemia test (TTI);
- at 11 patients IGF1 had a low value, while at 13 children the value was normal.

Mutations in the region of the GH receptor gene that codify the extracellular domain of the receptor were identified at 4 from the 24 studied children. One of the four children had a complex heterozygous conformation, with a mutation that decreases the receptor affinity for GH and another mutation that can influence the function of the receptor, other than ligand binding. The other three children had a single mutation in one of the gene alleles. One of these introduces a premature termination codon, while the other two cause the substitution of one single amino acid in the conserved structural domain of the receptor.

From the DNA samples of the patient no. 1 (G.D.), one of the PCR genomic fragments is anomalous. This exon codifies the extracellular domain of the GH receptor and we did not find other abnormalities which would be able to affect this domain.

DNA of patient 2 (V.M.) shows an anomalous strip in a PCR product of exon 5. We did not notice any other mutations at this patient. The most frequent mutation in exon 5 was noticed in position 418, where it is put a stop codon instead of the cysteine from position 122 (Cys122Stop). This mutant allele is probably a “null mutation” because it does not produce a functional protein. As the protein synthesis stops at this point we can not conclude if this patient is monozygous or heterozygous.

Patient 4 (C.V.) had abnormal strips in exons 4 and 6. This child is a complex heterozygous with a mutation in exon 4 as well as in exon 6. These two mutations were noticed in different sub clones that cover regions from exon 4 to exon 6.

The patient 7(S.A.), like patients 1 and 4 shows the alteration of a single allele.

After a year of treatment with genetic recombinent growth hormone, all the 24 children with ISS had an improvement of the average of the Z score of the height from -3.24 to -2.44 SD.

Out of the 4 children with GHR mutations, 3 knew an improvement of the Z score of the height under treatment, while the patient S.A. aged 5.4 years the pituitary dwarfism emphasized from -3.20SD to -3.49 SD.
The patient 4 (C.V.), aged 6.11, even if he had a complex heterozygous conformations with abnormal strips in exons 4 and 6, the growth speed under treatment was of 8 cm/year, comparatively with V.M. and S.A., who showed alteration of a single allele, but whose growth during the therapy was of only 5 cm/year, respectively 4 cm/year.

**Chapter IV. CONCLUSIONS**

1. Today, it is well-known that there is a huge spectrum of secretion and discharging the growth hormone, from the children with GH deficiency to the children with short stature, but having a normal somatotrop ax.

2. The somatotrop deficiency for a long period, besides the consequences on height and the intermediary metabolisms, has unfavorable consequences over the mineral bone density, over the cardiac function, the cognitive and the memory functions. This thing motivates an early diagnose of growth hormone deficiency, on the base of well-established criteria, in order to begin the treatment.

3. It is important for obtaining a good height that the therapy of substitution with the growth hormone should begin before puberty starting, known-being the fact that the gonad hormones accelerate the bone maturation. At the patients with somatotrop deficiency who we studied, 71.19% of the patients were of prepubertal age.

4. The conclusions after a year of treatment with rhGH underline a significant improvement of the growth speed, comparatively with the year prior the beginning of the therapy, as well as an improvement of the short stature with 0.74SD. The growth speed under treatment and the Z score of the height had comparable values to boys and to girls.

5. In the group we studied, from the 59 children with GHD, 12 patients (20.37%) associated subclinical hypothyroidism, while one of them was diagnosed with chronic autoimmune thyroiditis. In the case of these children, the treatment with thyroid hormones in moderate doses should be associated with the growth hormone, because T4 multiplies the receptors for GH.

6. It was proved that there are different variables that correlate, in a negative or in a positive way, with the answer to the therapy with rhGH at the children with GHD. The obtained results show correlations between the growth speed during the treatment and the following parameters: the chronological age at the beginning of the
treatment, the bone age, the delay of the bone age with more than 3 years.

7. At the patients with Turner syndrome it is important to administrate GH for stimulating the growth, even if the improvement of the height is not spectacular, at the puberty age the estrogen-progestin substitution should be added in order to develop and to maintain the sexual characters.

8. After a year of rhGH treatment our patients with idiopathic short stature showed an improvement of the statuary deficiency with an average of 0.8 SD.

9. We have to mention the fact that the syndrome of GH partial insensitivity can be a rare cause of so-called idiopathic short stature with a normal dynamics of GH stimulation tests, but taking into account the weak results obtained after the tests for IGH generation, molecular analyses of the GH receptor and its functional studies should be performed.

10. In our subgroup of 24 children with idiopathic short stature, we made molecular analyses regarding SSC polymorphism of the GHR gene and we identified 4 mutations in the region of the gene that codifies the extracellular domain of the GH receptor.

11. It is difficult to appreciate if the molecular flaws we identified are responsible for the weak answer to the rhGH therapy at 3 patients or this fact is due to other factors.

12. The genetic analyses brought an additional dimension in investigating the short stature, but, anyway, the nature of the particular mutations is not a prediction factor of the answer to the specific therapy for growth promoting. The identification of a specific genetic mutation also has a great importance in clarifying the etiology of growth deficiency.

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