HIGH GROWTH HORMONE LEVELS IN CLINICALLY SHORT STATURE CHILDREN

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Background: Growth Hormone (GH) is secreted from the anterior pituitary gland. It binds to receptors on the surface of target cells, stimulates production of Insulin-like growth factor-I (IGF-I) leading to growth of almost all tissues of the body capable of growing. Growth failure (height below 3rd centile) occurs in children who do not secrete sufficient amount of GH. In some children, however, short stature is present in the presence of high levels of GH in their blood and they also secrete normal to increased amounts of GH in response to stimulation tests when tested for possible deficiency of GH. This condition is known as GH resistance syndrome or Larons syndrome (LS). **Methods:** All patients after a thorough clinical evaluation underwent GH evaluation protocol as follows. On arrival in the lab a blood sample was collected for basal GH level in each patient. Screening was performed by subjecting the patients to exercise stimulation test and/or L-dopa stimulation test. Patients with GH deficiency underwent insulin tolerance test (ITT) after one week for confirmation. All the basal and post-stimulation samples were analyzed for GH levels. A level below 10mIU/L indicated GH deficiency, between 10-20mIU/L as borderline and an adequate response was defined as a GH >20mIU/L. Patients with a basal GH level of >20mIU/L and/or a post-stimulation level of >40mIU/L were arbitrarily considered as having exaggerated GH levels. This article evaluates the high plasma growth hormone levels among clinically short stature children undergoing growth hormone stimulation tests. **Results:** Two hundred ninty-three patients reported for GH evaluation. Twenty were excluded for various reasons. Thus 273 patients were included for GH evaluation out of which 66(24.2%) showed GH deficiency, 89(32.6%) were borderline while 118(43.2%) patients exhibited adequate response, with GH levels of >20mIU/L. A number of patients unexpectedly showed very high GH levels on screening tests. Out of 118 patients, 21 showed either very high basal levels of >20mIU/L and/or a much-exaggerated response to stimulation tests with levels more than about 40mIU/L. Close consanguinity was found in 67% of patients showing very high GH levels. **Conclusion:** Some children with idiopathic short stature may show high levels of GH during their evaluation for GH deficiency. We identified a considerable number of such patients. These patients require further investigations.

KEY WORDS: Growth Hormone, Resistance Syndrome, Short Stature, Exercise, L-dopa, Insulin Tolerance Test (ITT).

INTRODUCTION

GH is secreted from the anterior pituitary gland in the form of pulses throughout the day and night. These bursts of secretion increase the total daily GH secretion during the periods of maximal growth in adolescence. A substantial part (20-40%) of total 24h secretion of GH occurs during the first 90 minutes of nocturnal sleep.[1,2]

During the day GH secretions and hence the serum levels are influenced by exercise, stress and nutrient intake. Due to its pulsatile secretion and a short half life of 10 minutes, the basal level alone has no role in the diagnosis of GH deficiency. Therefore dynamic function tests, which include various types of physiological and pharmacological stimuli, are necessary for evaluation of GH response in an individual.[3-5]

Like other peptides and protein hormones GH binds to the cell membrane associated receptors on the surface of target cells. The sites of action of GH are ubiquitous.[1] It exerts pleotropic effects on growth and metabolism and it causes growth of almost all tissues of the body that are capable of growing.[6] GH action requires dimerization of two receptors with a single GH moiety and subsequent activation of a complex cascade of tyrosine kinases. GH stimulates production of Insulin-like growth factor-I (IGF-I) after its attachment to the cell receptors.[4] IGF-I further exerts the actions characteristic of GH.

GH action is modified by the soluble serum growth hormone binding protein (GHBP) that is identical to the extra-cellular domain of the GH receptors. Majority of GH circulates normally in blood bound to these proteins.[7] Growth failure (height below 3rd percentile) occurs in children who do not secrete sufficient amount of GH. In some children, however, short stature is present in the presence of high levels of GH in their blood and they

also secrete normal to increased amounts of GH in response to pharmacologic stimulation.

Laron and colleagues were the first to describe short stature with characteristic features of isolated GH deficiency but with elevated serum levels of GH in these children.[8,9] The condition is now known as Laron Syndrome (LS). It is a familial disorder with an autosomal recessive form of inheritance.

The cause of growth failure in the majority of these children has remained unknown.[10] The condition was originally thought to result from an abnormal growth hormone. However subsequent studies have shown that these children have a defect in the ability of the target cells to respond to GH due to defective GH receptor.[6] Such a defect, indeed, could occur in either the GH receptor (type I) or the intra-cellular mediators of GH signaling (type II). Thus these children have been found to have low levels of serum growth hormone binding proteins (GHBP) and a failure to produce Insulin-like growth factor I (IGF-I) in the presence of very high circulating GH levels.[9,11]

MATERIALS AND METHODS

The study was carried out in the Department of Chemical Pathology and Endocrinology, AFIP Rawalpindi, during a period of one year from June 1999 to July 2000.

The study population consisted of 293 patients out of which only 273 short stature ambulatory children were evaluated for their GH secretion. All the patients underwent a routine evaluation including medical history and physical examination, although children had already been medically examined by the referring physicians To keep a record and define the selection criteria a pre-designed proforma, for each child, was filled in which included the name, age, sex, place of residence, perinatal history, height and weight, heights of siblings, parental heights, history of any medical treatment and performance at school. Patients having any chronic disease, dysmorphism, evidence of endocrine disease like hypothyroidism and Cushing's syndrome, and malnutrition, psychosocial deprivation or any evidence of delayed onset of puberty were excluded. Height and weight of each child were recorded on standard growth charts. Only patients with heights below 3rd percentile were included. For bone age determination, a radiograph of non-dominant hand and wrist was considered essential.

The initial screening of GH was carried out by subjecting the patients to Exercise Stimulation Test and L-dopa Stimulation Test.

After an overnight fast, a basal blood sample for GH was collected, and each child performed a 20 minutes exercise on a treadmill under direct supervision of an attending doctor. On completion of exercise a venous blood sample was collected. Now L-dopa stimulation test was carried out by administering tablet L-dopa orally along with routine breakfast. The dose of L-dopa was 125mg for a child weighing < 15 Kg, 250mg for15 to 30 Kg and 500 mg for > 30 Kg. A blood sample was collected after 90 min of L-dopa administration. Patients who showed GH deficiency on screening tests were subjected, after a gap of 1 week, to insulin tolerance test (ITT).

All the blood samples for GH estimation were collected in disposable plastic syringes. Serum was separated in disposable sterilized, plain plastic tubes and stored at –20C till analyzed for GH.

Serum samples were analyzed for GH on "IMMULITE", an Automated Immunoassay Analyzer of Diagnostic Products Corporation (DPC), USA. The assay is based on the principle of a solid phase two-site chemiluminescence enzyme immunometric assay. It calculates test results for controls and patient samples from the observed signals, using a stored Master Curve, and generates a printed report. The assay has a working (reportable) range of GH from 0.13 to 104mIU/L. The intra-assay and inter-assay coefficient of variability for this kit at the decision limits were 2.5% and 3.8%, respectively.[12]

Individual investigators have used their own test protocols and arbitrary cut off values for test results.13 In our setup all patients with basal and post-stimulation GH levels of <10mIU/L were regarded as GH deficients, between 10 to 20mIU/L as borderline cases, while those with a GH level of >20mIU/L in any of the blood samples were considered as having adequate GH reserves.

Patients showing either very high basal levels of >20mIU/L and/or a post stimulation GH level more than 40mIU/L were arbitrarily regarded as those with unusually elevated GH levels.

Data was analyzed using SPSS version 11.0. Data was found to be non-normally distributed therefore results were expressed as median and range.

RESULTS

A total of 293 patients were referred for evaluation of GH deficiency. Twenty were excluded; eight had heights above 3rd percentile and twelve had an organic basis for their short stature. Thus only 273 patients were included for GH evaluation and data analysis. Out of 273 children 189 (69%) were males and 84 (31%) females with an age range of 2 years to 20 years.

Table-1: Patients with exaggerated GH responses (n=21)

Response	Basal Median (Range) mIU/L	Post exercise Median (Range) mIU/L	Post L-dopa Median (Range) mIU/L
Patients with high post-stimulation GH levels (n=14)	2.4 (1.0-12.0)	46.1 (37.7-74.0)	22.9 (11.5-100.0)
Patients with high basal+ high post-stimulation GH levels (n=7)	25.4 (20.0-53.0)	51.3 (37.8-68.0)	25.1 (14.5-37.0)

(GH response: basal >20mIU/L and/or post stimulation >40mIU/L)

On screening tests, 118(43.2%) patients showed a normal response with GH levels of >20mIU/L, a borderline response in 89(32.6%), while 66 patients (24.2%) exhibited GH deficiency with levels <10mIU/L.

Out of 118 patients exhibiting normal responses on stimulation tests a considerable number (n=21) comprised of those who had unexpectedly very high GH levels with either very high basal levels of >20mIU/L and /or a very exaggerated post-stimulation response with levels more than 40mIU/L. The data was found to be non-normally distributed therefore median and ranges were calculated. Out of these 21 patients, 14 had median basal GH levels of 2.4mIU/L but they showed a post-stimulation hyper-response and had median post exercise and post L-dopa GH levels of 46.1 and 22.9mIU/L respectively. The other 7 patients had median basal GH level of 25.4mIU/L. These patients also showed a hyper-response to exercise stimulation test with median GH of 51.3mIU/L, while the median post L-dopa stimulation GH was 25.1mIU/L. (Table-1)

Out of these 21 patients with elevated GH levels 14 were males (age range of 5-16.5years) and 7 were females (age range of 5-17years). All patients had proportionate short stature. In one case two sibs while in two cases each had one sib with short stature too. The parents were first cousins in 33.3%, second cousins in another 33.3%, while the remaining 33% were non-related.

DISCUSSION

Laron and colleagues in Israel were the first to describe short stature with the characteristic features of isolated GH deficiency but with elevated serum levels of GH in children.[8,9,11,14] The condition initially termed Laron-type dwarfism is currently known as Laron syndrome (LS), Growth hormone resistance syndrome, primary GH insensitivity (GHI), GH receptor insensitivity syndrome, or Laron's syndrome. Numerous independent populations of Laron syndrome patients have been identified in Europe, Africa, North and South America and Asia.

We found a considerable number of short stature children with either basal and/or post-stimulation very high GH levels. Some patients had low basal GH but very high post-stimulation GH levels, for example in a 12 years old male child post exercise and L-dopa stimulation GH levels rose to more than 74mIU/L and 100mIU/L respectively over basal level of 10mIU/L. While others had high basal GH levels and also showed a similar hyper-response on stimulation tests with marked rise of >40mIU/L in their GH levels especially after exercise stimulation test and a moderately high GH levels after L-dopa stimulation tests. This indicates release of maximum of the GH reserve during exercise stimulation test. Laron and coworkers, in various studies, have reported upon the dynamics of GH secretion in these patients. They found very high overnight fasting GH levels and noticed that nocturnal pulses in these patients may reach peak levels of 70-100mIU/L.[9]

This disorder phenotypically resembles GH deficiency. These patients may also demonstrate the metabolic consequences of GH deficiency including truncal obesity, delayed puberty and hypoglycemia despite normal or elevated concentrations of biologically active GH. Laron syndrome (LS), although, is traditionally associated with dysmorphic facial features however many GH resistant patients show normal facial appearance.[15] Besides genetic factors environmental factors are also thought to contribute to the large amount of variation in phenotype of these patients.[16] Our patients had no marked dysmorphic facial features but had a proportional short stature. Moreover none of the children had any known disease at the time of study. No child had evidence of malnutrition as judged by history and physical examination. The family psychosocial structures in all cases were clinically stable.

The etiology and characteristics of this disease have been extensively reviewed.[17,18] This condition was originally thought to result from an abnormal growth hormone molecule.[19,20] Later it was shown that these patients have defective GH receptors on the cell surface. The defect was first demonstrated by experiments showing the absence of binding of GH to hepatocytes of these patients.[8] Indeed more than 30 different mutations in the GH receptor gene have been identified ranging from exon deletions to nonsense, frameshift and splice and

missense mutations of exons and introns and majority are in the extracellular domain of the receptor.[9,15]

GHBP are the solubilized extracellular fragment/domain of the GH receptor. It has been found that in such patients the levels of GHBP are low. Determination of serum GHBP can be used as a simple quantitative estimation of the extracellular domain of GH-R. Its deficiency reflects the relative lack of cell surface GH receptors.[9,10,21]

GH leads to production of IGF-I after its binding with the target cells. IGF-I also acts as a negative feed back for GH secretion and its absence thus leads to still more secretion of GH from the somatotrophs of anterior pituitary gland.[4,22,23] These patients show very low serum IGF-I concentrations.[24] A somatomedin generation test and also GH treatment in these children do not show any rise in their serum IGF-I concentration or stimulate growth. This also indicates that this syndrome is due to abnormal or deficient cellular growth hormone receptors.[23,25,26] leading to end organ resistance to the hormone.

For about 20 years since its original description, Laron syndrome remained a very rare untreatable condition. However in late 1980s recombinant IGF-I became available and treatment with IGF-I was demonstrated to accelerate linear growth.[27] Effects of recombinant human IGF-I (rhIGF-I) on linear growth of children with receptor mutations have proven beneficial and it has been recommended as a long-term replacement therapy for patients with Laron's syndrome.[28] Identification of these children is therefore important. Our patients require further investigations and need IGF-I and GHBP-3 estimation but due to lack of facilities we could not perform these estimations.

During clinical evaluation, our patients were found to have no endocrine disturbances besides the GH anomaly. The number of male patients is more as compared to females, probably because of the fact that parents are more concerned about the overall health of their sons as compared to their daughters in our society. Close consanguinity, in these patients with elevated GH levels, has been reported in the literature.[8,11,29] This indicates that this type of dwarfism is inherited as an autosomal recessive disorder. There was close consanguinity (67%) among the parents of these children with high levels of GH.

In our study, the number of children with elevated GH levels appears fairly high. The condition initially described in Jewish communities and in patients of Mediterranean origin, is also not uncommon in our people. These patients require identification and further investigations. They need estimation of IGF-I levels before and after human GH administration for the confirmation of diagnosis and documentation. Serum assays for IGF-I and GHBP-3 are now commercially available and require to be included in the existing repertoire for the complete evaluation of such patients.

CONCLUSION

Some children with idiopathic short stature may show very high levels of GH during their evaluation for GH deficiency. In our study, a considerable number of children showed elevated GH levels. It is not an uncommon condition in our people. These patients require identification and further investigations like estimation of IGF-I levels in their blood.

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