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Evaluation of Insulin-Like Growth Factor-1 and Its Impact on Growth Hormone Therapy in Growth Hormone-Deficient Indian Children

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ABSTRACT

Background: Insulin-like growth factor-1 (IGF-1) and IGF-binding protein 3 (IGFBP-3) are used widely for evaluating growth hormone deficiency (GHD). We evaluated the effect of recombinant human growth hormone treatment on serum IGF-1 concentrations in Indian children with GHD over a period of 24 months.

Patients and Methods: Patients who presented with short stature were evaluated. The enrolled subjects exhibited a height standard deviation score (SDS) of less than -3 and/or a height velocity SDS of less than -2 over a 12-month period, and they displayed GH concentrations of less than 10 ng/ml in 2 provocative tests. Patients received a detailed physical examination that included auxology, pubertal staging, and biochemical assays to measure IGF-1 concentration. All patients received GH at a dose of 0.3 mg/kg/week in 7 divided doses subcutaneously daily at night. Patients with multiple pituitary hormone deficiencies received additional substitution therapy.

Results: Twenty-five prepubescent children (male:female = 14:11) at a mean age of 8.6 ± 2.9 years were enrolled. The height SDS at baseline, 1 year, and 2 years was -5.38 ± 1.4, -4.10 ± 1.4, and -3.6 ± 1.3, respectively ($P < 0.005$), whereas the IGF-1 SDS at baseline, 1 year, and 2 years was -3.40 ± 0.8, -1.74 ± 1.2 and, -1.54 ± 1.7, respectively ($P > 0.1$). No significant difference in height change SDS was detected between children with an IGF-1 SDS in the normal range and children with an IGF-1 SDS of less than -2 at 2 years. Bone age advancement, the occurrence of puberty, and levels of fasting glucose and HbA1C did not change during therapy.

Conclusions: Our study on Indian children indicates that changes in serum IGF-1 SDS concentrations may not be a reliable marker for responsiveness to GH therapy.

▸ *Implication for health policy/practice/research/medical education:*
Response to growth Hormone therapy in Indian children can not be edequately gauged by change in serum IGF-1 concentration SDS.

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1. Background

The major role of growth hormone (GH) during growth and development is promoting longitudinal bone growth. GH binds to the GH receptor and results in the release of polypeptides called insulin-like growth factors (IGFs), which mediate the growth promoting effects of GH (1). IGFs are critical for postnatal growth, the regula-

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tion of cell growth, cellular differentiation, apoptosis, and transformation (2). The incidence of GH deficiency (GHD) is believed to be about 1 in 4,000 to 1 in 10,000 live births. IGF-1 concentrations remain fairly constant throughout the day, and thus, serum IGF-1 is measured routinely to monitor GHD patients (3). Owing to the limitations of GH stimulation tests, a gradual shift has occurred in GHD diagnosis from GH-based approaches to those that utilize IGF (4). A lower concentration of IGF-1 has been detected in normal Indian children, compared to age- and sex-matched Western counterparts (5). Significant correlations between IGF-1 concentration and the height standard deviation score (SDS) have been reported in GHD children receiving GH treatment (6).

2. Objectives

Data on serum IGF-1 concentrations in Indian children with GHD and on the impact GH treatment has on IGF-1 concentrations is scarce. Here, we present data collected over a period of 24 months on the impact of recombinant human GH treatment on auxological and biochemical parameters (serum IGF-1 concentrations) in Indian children with GHD.

3. Patients and Methods

The study was conducted from January 2008 to March 2011, at the Department of Endocrinology at Medwin Hospital, Hyderabad, India. The hospital's ethical committee approved the study. Informed consent was obtained from the parents of each participant.

Children who presented with short stature in other departments also were enrolled. Patients were evaluated by a thorough examination of medical history, a physical examination with anthropometry, and pubertal staging. Standing height was measured using a portable stadiometer (Leicester height meter; Child Growth Foundation, UK; range, 60–207 cm). Weight was measured using an electric scale (Salter, India) accurate to 100 g. The Agarwal growth chart for boys and girls was used. Biochemical tests were used to rule out other causes of short stature. Bone age was measured for each patient by using plain radiographs of the left hand and the Greulich and Pyle method. Overnight fasting venous blood samples (5 ml) were collected in plain vacutainers with no anticoagulant (Becton Dickinson and Company, Franklin Lakes, NJ, USA) and used to measure serum GH and IGF-1 concentrations. Serum GH was measured by a solid-phase, 2-site chemiluminescent immunometric assay; the intra-assay coefficient of variation (CV) was -5.3% and the inter-assay CV was -5.5%. Serum IGF-1 was measured by a solid-phase, enzyme-labeled chemiluminescent immunometric assay; the intra-assay coefficient of variation (CV) was -5.8% and the inter-assay CV was -3.1%. The IGF-1 concentrations were converted into SDS (7). GHD was confirmed by using 2 different provocation tests (0, 30, 60, 90, and 120 min after subcutaneous administration of insulin [0.05 to 0.1 U/kg] and oral administration of clonidine [0.15 mg/m^2]). All patients were evaluated simultaneously for other pituitary hormone deficiencies.

3.1. Inclusion Criteria

- Height below the third percentile or 2 SDS below the national mean standards.
- Over a 24-month observation period, growth velocity of successive height measurements below the 25th percentile on the velocity chart.
- GH concentration of less than 10 ng/ml during 2 provocation tests.

3.2. Exclusion Criteria

- The presence of any active tumor, active systemic disease, chromosomal abnormality, or syndromic disease.
- Chronic treatment with any medication other than thyroid or cortisol replacement.

Patients were treated with recombinant human growth hormone (rhGH) at a dose of 0.3 mg/kg/week in 7 divided doses given subcutaneously at night on every weekday for 24 months. Patients with multiple pituitary hormone deficiency (MPHD) were maintained on a stable daily dose of levothyroxine (100 μg/m^2) and/or hydrocortisone (10 mg/m^2). Patients were evaluated at 3-month intervals. The examinations conducted included a complete physical examination, including auxological and pubertal assessment; biochemical assays for GH and IGF-1; bone age determination using plain radiographs; and measurement of HbA1C and fasting glucose levels.

3.3. Statistical Analysis

After meticulous examination of the original study forms, data were collected into a database in the mean ± standard deviation format. Online Graphpad Quickcalc software was (http://www.graphpad.com/quickcalcs/index.cfm) used to perform statistical analyses. Analysis of variance (ANOVA) and the unpaired *t* test were used to calculate differences among groups. P value < 0.05 was considered significant.

4. Results

In total, 25 GHD children (male:female = 14:11) with a mean age of 8.6 ± 2.9 years, were enrolled in the study. All patients were in the prepubertal stage (Tanner stage 1) with a bone age of less than 8 years for girls and less than 9 years for boys at the time of enrollment. Twenty-one subjects presented with isolated GHD and 9 with MPHD. The anthropometric and biochemical characteristics of GHD children at baseline and 12 and 24 months are shown in *Table 1*. No significant differences in height SDS were observed between children with an IGF-1 SDS in the normal range and children with an IGF-1 SDS of less than -2 at the conclusion of the 24-month observation period. The mean changes in height and the percentages of children with normal IGF-1 levels is shown in *Table 2*.

Table 1. : Anthropometric and Biochemical Characteristics of GHD Children at Baseline and 12 and 24 Months

	Time, mo, mean ± SD		
	Baseline	**12**	**24**
Height, cm	98.6 ± 14.3 [a]	110.7 ± 16.1 [a]	117.9 ± 16.2 [a]
Height SDS	-5.38 ± 1.4 [a]	-4.10 ± 1.4 [a]	-3.6 ± 1.3 [a]
BMI, kg/m^2	14.7 ± 1.8	14.8 ± 2.8	15.9 ± 3.0
BMI SDS	-1.2 ± 0.6	-1.5 ± 1.7	-0.9 ± 1.4
Growth velocity SDS	-2.1 ± 1.1 [a]	3.3 ± 1.9 [a]	2.1 ± 1.3 [a]
Peak GH, ng/ml	0.97 ± 1.2	–	–
Serum IGF-1, ng/ml	29.2 ± 17.3 [a]	121.2 ± 113.8 [a]	195.9 ± 147.1 [a]
IGF-1 SDS	-3.40 ± 0.8	-1.74 ± 1.2	-1.54 ± 1.7

[a] $P < 0.05$ is statistically significant.

Table 2. Mean Change in Height and IGF-1 Levels in Normal Range (% Age) on Therapy

	Mean Change in Height, cm	IGF-1 Levels in Normal Range, %
6 mo	6.8	10
12 mo	5.8	23
18 mo	4.1	30
24 mo	4.2	47

Table 3. Effect of GH Treatment on Biochemical Parameters and Skeletal and Pubertal Maturation.

	Time, mo, mean ± SD		
	Baseline	**12**	**24**
FBS, mg/dl	83.3 ± 15.1	85.6 ± 16.9	86.1 ± 17.1
HbA1C, %	5.3 ± 0.5	5.4 ± 0.6	5.3 ± 0.4
Bone age delay, y	2.9 ± 1.2	2.8 ± 1.1	2.7 ± 1.4
Advancement of bone age, y	-	1.2 ± 1.0	0.9 ± 0.8
Subjects reach puberty	-	1	2
Male	-	0	0
Female	-	1	2

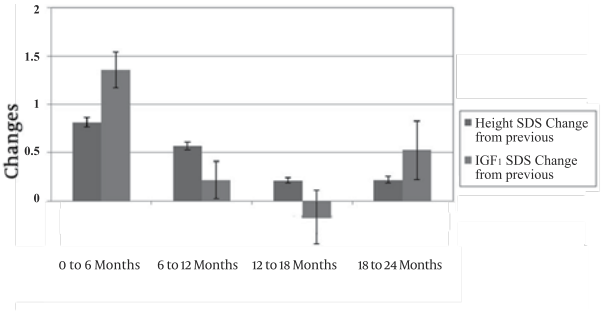

Figure 1. Change in Height SDS and IGF SDS at 6-Month Intervals 4 (6, 12, 18, and 24 Months).

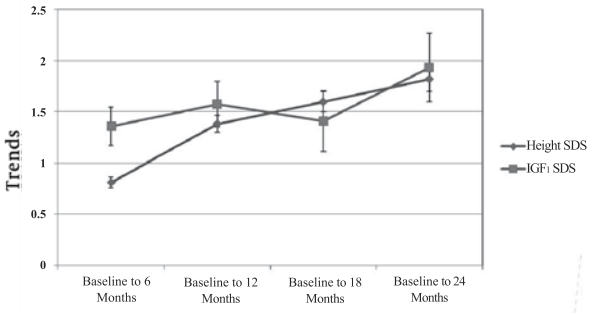

Figure 2. Trends in Height SDS (Quadratic Curve) and in IGF-1 SDS (Exponential Curve) Changes from Baseline to 6, 12, 18, and 24 Months.

No significant differences were observed in height velocity (HV) SDS at 12 and 24 months for subjects with either normal IGF-1 (-1.5 ± 1.2 and -0.9 ± 1.7, respectively) or an IGF-1 SDS of less than -2 (-1.5 ± 1.8 and -0.9 ± 1.1, respectively). The changes from baseline of the IGF-1 SDS and the height SDS were tested at 6-month intervals (6, 12, 18, and 24 months). Using repeated-measures ANOVA, we found that the change in IGF-1 SDS did not reach statistical significance at any time point ($P > 0.1$). However, the change in height SDS did differ significantly from the baseline ($P < 0.0001$). The changes in height SDS and IGF-1 SDS that were observed at the last 4 time points (6, 12, 18, and 24

months) are depicted in *Figures 1* and *2*. No instances of pseudotumor cerebri, worsening scoliosis, leukemia, or slipped capital femoral epiphysis were reported. The parents were counseled extensively, and thus, the drop-out rate in our study was 0. General laboratory parameters, including chemistry panels and erythrocyte sedimentation rate, did not display significant abnormalities at any point.

5. Discussion

Since Raben's landmark report in 1958 on the successful height gain of a GHD patient in response to GH that was extracted from human pituitary glands, the use of GH therapy has become a standard clinical practice for treating growth disorders (8). Until recently, little data had been published on the expected magnitude of the changing response variables resulting from GH treatment of different growth disorders (9). Informed growth response predictions are useful for monitoring the effects of GH treatment. Because it is the strongest indicator of future height outcomes, growth during the first year of GH treatment in prepubertal children is particularly important and should be optimized (10). In this study, we evaluated the impact of GH treatment on IGF-1 levels in Indian children. IGF-1 and IGF-binding proteins (IGFBPs) are used widely to evaluate and diagnose GHD (11). The IGFs are related GH-dependent peptide factors that are believed to mediate many anabolic and mitogenic actions of GH. The serum level of the major GH-dependent peptide IGF-1 is stable during the day, mainly because of the complex formed by IGF peptides with a family of IGFBPs (12). The potential to assess GH status with a single estimation of circulating IGF-1 levels suggests that dynamic GH provocation tests may become unnecessary. Initially, difficulties in IGF assays arose because of interference caused by the presence of IGFBPs (12). This issue has been overcome by a variety of approaches, including acid size-exclusion chromatography before conducting the IGF assay, and blocking IGFBP-binding sites with an excess of the non-specific IGF peptide (excess IGF-2 for an IGF-1 assay) or IGF analogs that do not bind to IGFBPs as radioligands (13).

Of the 6 known IGFBPs, IGFBP-3 is the major serum carrier of IGF peptides (14). IGFBP-3 circulates as part of a ternary complex of IGFBP-3, an IGF peptide, and an acid-labile subunit (15). Both the acid-labile subunit and IGFBP-3 are GH dependent. Since IGFBP-3 levels reflect combined IGF-1 and IGF-2 concentrations, the age dependency of IGFBP-3 is less striking than that of IGF-1. Similarly, nutritional status has less influence on IGFBP-3 levels than on IGF-1. The IGFBP-3 assays, which do not require the separation of IGFBPs from IGF peptides, are technically easier to perform. Some have speculated that the level of IGF-1 achieved by the individual patient has a higher impact on results than the GH dose received by the patient (16). As per the recommendation of the Growth Hormone Research Society Consensus Guidelines statement on the diagnosis and treatment of GHD in childhood and adolescence (17), measured IGF-1 correlates with the growth response, and patients with subnormal IGF-1 levels tend to grow less rapidly; this suggests that such patients may benefit from a higher IGF-1 concentration. However, the use of IGF-1 to diagnose GHD in children is controversial (18) because of several factors, including hepatic production of IGF-1 altered by nutritional factors, ethnicity, age, pubertal status, gonadal steroids, and a host of other factors like diabetes mellitus, renal failure, and liver failure (19). Several researchers have indicated that although serum IGF-1 is a poor surrogate marker for tissue IGF-1 (20), it may function as a tool for optimizing GH therapy. The sensitivity and specificity of these tests are variable (11). The response to GH therapy has been expressed traditionally in terms of HV (measured as centimeters/year) and derived from the data observed over the period of a year. In general, the average HV of healthy children decreases as they approach puberty, indicating that direct numerical comparison between HV in differently aged children is not possible. The most commonly used HV reference standards (21), which represent growth in healthy children, are based on longitudinal measurements collected from relatively small cohorts. Bakker *et al*. (9) have shown that while treating GHD children with GH, the HV SDS derived from Tanner reference data increase up to 9 years of age and then decrease, suggesting that HV SDS is probably not adequate to describe GH-induced growth, particularly if the pubertal stage is not considered in the reference data. If the HV data are available from the pretreatment year, then the response to GH could be expressed as HV or HV SDS. In our study group, the availability of pretreatment HV data allowed for easier calculations of posttreatment HV SDS. The response to GH can be expressed with higher confidence in terms of height SDS (22), and this is because the means and SD values from normative reference heights are robust, owing to the large number of individuals included in cross-sectional studies (in contrast to the number of patients investigated in longitudinal HV studies). In addition, the Tanner and Prader height references (means and SD values) (21) have been adapted for evaluating children with delayed puberty (23). However, the fact that the mean height for age and sex vary according to the underlying cohort used to derive the standard references needs to be considered (24). Michael *et al*. estimated a height SDS of less than 0.40 in patients with severe GHD to be an inadequate response to GH during the first year. In patients with less-severe GHD, girls with Turner Syndrome (TS), or children born Small for gestational age (SGA), a height SDS of less than 0.30 was deemed an inadequate response to GH during the first year (22). In our study group, significant improvement in height and height SDS was observed at all intervals. The improved height following GH treatment is affected by several factors. The maximum growth response occurs during the

initial years: 10–12 cm/year during the first year and 7–9 cm/year during the second and third years (25). The rise in both parameters reached a maximum during the first year, with mean height growth of 12 cm in the first year and 7.5 cm in the second year.

For assurance of compliance, dosing, and safety considerations, yearly monitoring of serum IGF-1 and IGFBP-3 levels is useful, particularly because of the association between elevated serum IGF-1 levels and certain cancers (26). For patients who display a suboptimal growth response or in whom the IGF levels remain low despite assurance of compliance with the injection schedule, increasing the GH dose within the U S Food and Drug Administration (FDA)-approved dose guidelines is reasonable. Dose reductions should be considered after the first 2 years of therapy in patients with serum IGF-1 levels substantially higher than normal. Continued treatment is generally futile if no increases in growth rate or serum IGF concentration over baseline are detected within the first 6 to 12 months in a compliant patient who is receiving an appropriate dose of GH (27). In a previous study, 14 patients were monitored for 2 years for growth, IGF-1 SDS, and IGFBP-3 SDS—all scores were significantly higher in those who received 50 μg/kg daily compared to 25 μg/kg (6). The dose response effect for GH persisted at a daily dose of 100 μg/kg for IGF-1, but not for IGFBP-3. They observed a different dose–response curve for growth induction, IGFBP-3 generation, and IGF-1 generation, and the effect reached saturation for growth first, for IGFBP-3 second, and for IGF-1 last.

The serum IGF-1 concentrations at 12 and 24 months improved significantly in our group of patients, but no significant differences in height SDS and HV SDS were observed at 24 months between children with IGF-1 SDS in the normal range and children with an IGF-1 SDS of less than -2. Other randomized trials have shown that measuring the response to GH as the cumulative height SDS achieved at 2 years of therapy correlated with both the IGF-1 SDS and IGFBP-3 SD scores during therapy (28). *Figure 1* shows that the maximal height change SDS and IGF-1 SDS occurred during the first 6 months of therapy. Thereafter, the increases in height SDS were lesser in magnitude, but they were positive for 24 months (-5.4 to -3.6). Between 12 and 18 months, however, IGF-1 levels exhibited a small non-significant dip (-3.4 to -1.5). No significant correlations were detected between the changes in height SDS and in IGF-1 SDS at the remaining time points ($P > 0.1$). GH is associated with acute transient insulin-like effects, but chronic GH exposure leads to decreased glucose utilization, increased lipolysis, and tissue refractoriness to the acute insulin-like effects of GH; this leads to a state of insulin resistance (29). In our group, glucose and HbA1C levels remained unchanged after treatment (*Table 3*). Bone age advancement and the occurrence of puberty did not change during therapy. Previous studies have suggested that the integration of parameters such as IGF-1 and IGFBP-3 concentrations, rather than weight-based GH dosage (6), leads to the design of improved individualized treatment plans (30). Similar responses have been found by Japanese and European investigators (31). The limitations of our study include study brevity, the use of a single center with only 2 years of follow-up, the relatively low number of patients, and the inclusion of patients from a limited geographical area surrounding the hospital. Previous studies have highlighted that measures of body composition, bodily function, and metabolic changes should all be taken under consideration when determining the best GH therapy regimen for children of short stature (22). Our study suggests that the change in serum IGF-1 SDS concentrations in Indian children that result from GH treatment may not function as an adequate marker for GH responsiveness. Future multicentric studies that include a higher number of patients with longer follow-up would be a more suitable option to evaluate the utility of monitoring IGF-1 and optimizing individual doses. These studies would also help in deciding whether these approaches will lead to a better response and fewer long-term complications.

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References

1. Daughaday WH, Rotwein P. Insulin-like growth factors I and II. Peptide, messenger ribonucleic acid and gene structures, serum, and tissue concentrations. *Endocr Rev*. 1989;**10**(1):68-91.
2. Lupu F, Terwilliger JD, Lee K, Segre GV, Efstratiadis A. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. *Dev Biol*. 2001;**229**(1):141-62.
3. Clemmons DR. Consensus statement on the standardization and evaluation of growth hormone and insulin-like growth factor assays. *Clin Chem*. 2011;**57**(4):555-9.
4. Rosenfeld RG. Is growth hormone deficiency a viable diagnosis? *J.Clin Endocrinol.Metabo*. 1997;**82**(2):349.
5. Dehiya RK, Bhartiya D, Kapadia C, Desai MP. Difference exist in circulatory levels of IGF-1 in Indian compared to Western age-matched population. *Indian Pediatrics*. 2000;**37**:990-7.
6. Kamp GA, Zwinderman AH, Van Doorn J, Hackeng W, Frolich M, Schonau E, et al. Biochemical markers of growth hormone (GH) sensitivity in children with idiopathic short stature: individual capacity of IGF-I generation after high-dose GH treatment determines the growth response to GH. *Clin Endocrinol (Oxf)*. 2002;**57**(3):315-25.
7. Juul A, Main K, Blum WF, Lindholm J, Ranke MB, Skakkebaek NE. The ratio between serum levels of insulin like growth factor (IGF) I and the IGF binding proteins (IGFBP 1, 2 and 3) decreases with age in healthy adults and is increased in acromegalic patients. *Clin endocrinol*. 1994;**41**(1):85-93.
8. Raben MS. Treatment of a pituitary dwarf with human growth hormone. *J Clin Endocrinol Metab*. 1958;**18**(8):901-3.
9. Bakker B, Frane J, Anhalt H, Lippe B, Rosenfeld RG. Height velocity targets from the national cooperative growth study for first-

year growth hormone responses in short children. *J Clin Endocrinol Metab*. 2008;**93**(2):352-7.
10. Reiter EO, Price DA, Wilton P, Albertsson-Wikland K, Ranke MB. Effect of growth hormone (GH) treatment on the near-final height of 1258 patients with idiopathic GH deficiency: analysis of a large international database. *J Clin Endocrinol Metab*. 2006;**91**(6):2047-54.
11. Juul A, Kastrup KW, Pedersen SA, Skakkebaek NE. Growth hormone (GH) provocative retesting of 108 young adults with childhood-onset GH deficiency and the diagnostic value of insulin-like growth factor I (IGF-I) and IGF-binding protein-3. *J Clin Endocrinol Metab*. 1997;**82**(4):1195-201.
12. Rosenfeld RG, Lamson G, Pham H, Oh Y, Conover C, De Leon DD, et al. Insulinlike growth factor-binding proteins. *Recent Prog Horm Res*. 1990;**46**:99-159; discussion 159-63.
13. Rosenfeld RG, Gargosky SE. Assays for insulin-like growth factors and their binding proteins: Practicalities and pitfalls. *J Pediatr*. 1996;**128**(5, Supplement 1):S52-S7.
14. Martin JL, Baxter RC. Insulin-like growth factor-binding protein from human plasma. Purification and characterization. *J Biol Chem*. 1986;**261**(19):8754-60.
15. Baxter RC. Characterization of the acid-labile subunit of the growth hormone-dependent insulin-like growth factor binding protein complex. *J Clin Endocrinol Metab*. 1988;**67**(2):265-72.
16. Tillmann V, Buckler JM, Kibirige MS, Price DA, Shalet SM, Wales JK, et al. Biochemical tests in the diagnosis of childhood growth hormone deficiency. *J Clin Endocrinol Metab*. 1997;**82**(2):531-5.
17. Consensus guidelines for the diagnosis and treatment of growth hormone (GH) deficiency in childhood and adolescence: summary statement of the GH Research Society. GH Research Society. *J Clin Endocrinol Metab*. 2000;**85**(11):3990-3.
18. Yadav S, Krishnamurthy S. Insulin like growth factors and growth hormone deficiency. *Indian Pediatrics*. 2007;**44**(5):349.
19. Monzavi R, Cohen P. IGFs and IGFBPs: role in health and disease. *Best Pract Res Clin Endocrinol Metab*. 2002;**16**(3):433-47.
20. Yakar S, Liu J-L, Stannard B, Butler A, Accili D, Sauer B, et al. Normal growth and development in the absence of hepatic insulin-like growth factor I. *Proc Nat Acad Sci*. 1999;**96**(13):7324-9.
21. Tanner JM, Whitehouse RH, Takaishi M. Standards from birth to maturity for height, weight, height velocity, and weight velocity: British children, 1965. II. *Arch Dis Child*. 1966;**41**(220):613-35.
22. Ranke MB, Lindberg A. Observed and predicted growth responses in prepubertal children with growth disorders: guidance of growth hormone treatment by empirical variables. *J Clin Endocrinol Metab*. 2010;**95**(3):1229-37.
23. Ranke MB, Price DA, Reiter EO. *Growth hormone therapy in pediatrics: 20 years of KIGS*. S Karger Pub; 2007.
24. Willey B, Griffiths P, Cameron N, Norris S, Pettifor J. Socio-economic status and social support as predictors of infants being born small for gestational age in urban South Africa. In: Nicoletti I, Benso L, Gilli G, eds. Human Growth in Sickness and in Health: Plenary Lectures from the 10th International Congress of Auxology. Frienze: Edizioni Centro Studi Auxologici; 2006.
25. Price D, Ranke MB. Final height following growth hormone treatment; in Ranke MB, Gunnarsson R (eds): Progress in Growth Hormone Therapy - 5 Years of KIGS. Mannheim, . *J&J Verlag*. 1994:129-44.
26. Fine RN, Sullivan EK, Kuntze J, Blethen S, Kohaut E. The impact of recombinant human growth hormone treatment during chronic renal insufficiency on renal transplant recipients. *J Pediatr*. 2000;**136**(3):376-82.
27. Wilson TA, Rose SR, Cohen P, Rogol AD, Backeljauw P, Brown R, et al. Update of guidelines for the use of growth hormone in children: the Lawson Wilkins Pediatric Endocrinology Society Drug and Therapeutics Committee. *J Pediatr*. 2003;**143**(4):415-21.
28. Mandel SH, Moreland E, Rosenfeld RG, Gargosky SE. The effect of GH therapy on the immunoreactive forms and distribution of IGFBP-3, IGF-I, the acid-labile subunit, and growth rate in GH-deficient children. *Endocrine*. 1997;**7**(3):351-60.
29. Cutfield WS, Wilton P, Bennmarker H, Albertsson-Wikland K, Chatelain P, Ranke MB, et al. Incidence of diabetes mellitus and impaired glucose tolerance in children and adolescents receiving growth-hormone treatment. *Lancet*. 2000;**355**(9204):610-3.
30. Johannsson G, Rosen T, Bengtsson BA. Individualized dose titration of growth hormone (GH) during GH replacement in hypopituitary adults. *Clin Endocrinol (Oxf)*. 1997;**47**(5):571-81.
31. Yokoya S, Araki K, Igarashi Y, Kohno H, Nishi Y, Hasegawa Y, et al. High-dose growth hormone (GH) treatment in prepubertal GH-deficient children. *Acta Paediatr Suppl*. 1999;**88**(428):76-9.