RESEARCH ARTICLE

Etiological study of short stature in children and role of insulin like growth factor-1 and insulin like growth factor binding protein-3 as screening markers for growth hormone deficiency

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Abstract

Objective: To determine aetiological factors in children with short stature, and to evaluate the role of insulin like growth factor-1 and insulin like growth factor binding protein-3 as screening markers for growth hormone deficiency.

Method: The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from August 2020 to July 2021, and comprised children with short stature. Evaluation protocol included complete history and examination, baseline laboratory investigations, X-ray for bone age and karyotyping. Growth hormone status was assessed using growth hormone stimulation tests, and serum insulin like growth factor-1 and insulin like growth factor binding protein-3 levels were also assessed. Data was analysed using SPSS 25.

Results: Of the 649 children, 422(65.9%) were boys and 227(34.9%) were girls. The overall median age was 11 years (interquartile range: 11 years). Of the total, 116(17.9%) children had growth hormone deficiency. Familial short stature was present in 130(20%) children and constitutional delay in growth and puberty in 104(16.1%). There was no significant difference in levels of serum insulin like growth factor-1 and insulin like growth factor binding protein-3 in children who had growth hormone deficiency and those who had other causes of short stature ($p>0.05$).

Conclusion: Physiological variants of short stature were found to be more common in the population followed by growth hormone deficiency. Serum insulin like growth factor-1 and insulin like growth factor binding protein-3 levels alone should not be used to screen children with short stature for growth hormone deficiency.

Keywords: Short stature, Growth hormone deficiency, Growth hormone stimulation tests, IGF-1, IGFBP-3.

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Introduction

Concerns about their child's height is one of the most common causes for which parents seek consultation with a paediatric endocrinologist. It has psychosocial effects on both the parents and the children.[1] Short stature has been defined as an individual's height lower than the 3rd percentile or 2 standard deviations (-2SD) for the age and gender. It results from a complex mechanism involving a multitude of genetic, endocrine, systemic, environmental and nutritional factors. The aim of the assessment of short children is to identify the pathological causes of short stature so that appropriate treatment can be given timely.[2] A number of diseases must be first excluded in children presenting with short stature, before labelling them as growth hormone (GH) deficient.[3]

Approximately 2.5-3% of children across the globe are short.[4] The prevalence of short stature in Pakistan has been reported as 16.5% in children aged 6-12 years.[5] Every individual has a genetic potential to reach a certain height which is governed by many factors in the prenatal and postnatal periods. A precision diagnosis is not established in 50-90% of short children in spite of extensive clinical and laboratory investigations.[6] The axis between GH and insulin-like growth factor 1 (IGF-1) plays a key role in the development of normal musculoskeletal system and height.[7]

Familial short stature (FSS) and constitutional delay in growth and puberty (CDGP) are the most common physiological variants of short stature, which constitutes idiopathic short stature (ISS). ISS is the state in which the height of the person is lower than the standard 2SD for that age and gender in the absence of any systemic, endocrine, nutritional or chromosomal abnormality.[8] Pathological variants of short stature result from a wide variety of underlying disorders, which must be investigated and treated timely. Aetiological evaluation has a pivotal role in identification of physiological and pathological factors resulting in short stature. While physiological short stature needs only parental reassurance and follow-up, pathological short stature needs timely recognition and treatment.

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Data regarding different aetiologies of short stature in Pakistani children is limited. In contrast to western world, various factors, like environmental, genetic, nutritional deficiencies and infectious diseases, may be the underlying aetiology of short stature in the country. Measurement of serum IGF-1 is also considered important in growth hormone deficiency (GHD) screening. Many studies have assessed the role of IGF-1 in the diagnosis of GHD, but the results are not comparable.[9,10] The current study was planned to identify the aetiological factors responsible for short stature in Pakistani children, and to evaluate the role of serum levels of IGF-1 and IGFBP-3 as screening markers of GHD.

Subjects and Methods

The cross-sectional study was conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from August 2020 to July 2021. After approval from the institutional ethics review committee, the sample was raised from among those who got referral of endocrine clinic for the evaluation of short stature from different hospitals and clinics across Pakistan. Non probability convenient sampling technique was used. By using WHO sample size calculator[11] and keeping prevalence of GHD 16.5%5 in Pakistan, sample size calculated was 212. We included all 649 children who reported to us during the study period fulfilling inclusion criteria. All children aged <18 years of either gender with height -2SD or 3rd percentile for age and gender, and height velocity <4 cm/year were included. Evaluation protocol was based on the guidelines of the Growth Hormone Research Society (GHRS)[10] and included comprehensive history-taking, including medical history, history of consanguinity and family history, and physical examination which included phenotypic characteristics, head circumference, body proportions and pubertal staging. Birth history was taken for any perinatal complications and foetal growth abnormalities. Information was gathered regarding any past illness, chronic diseases, medicines used, nutritional status and milestones development. The diagnostic approach was charted out in detail (Figure).

Laboratory investigations included complete blood count

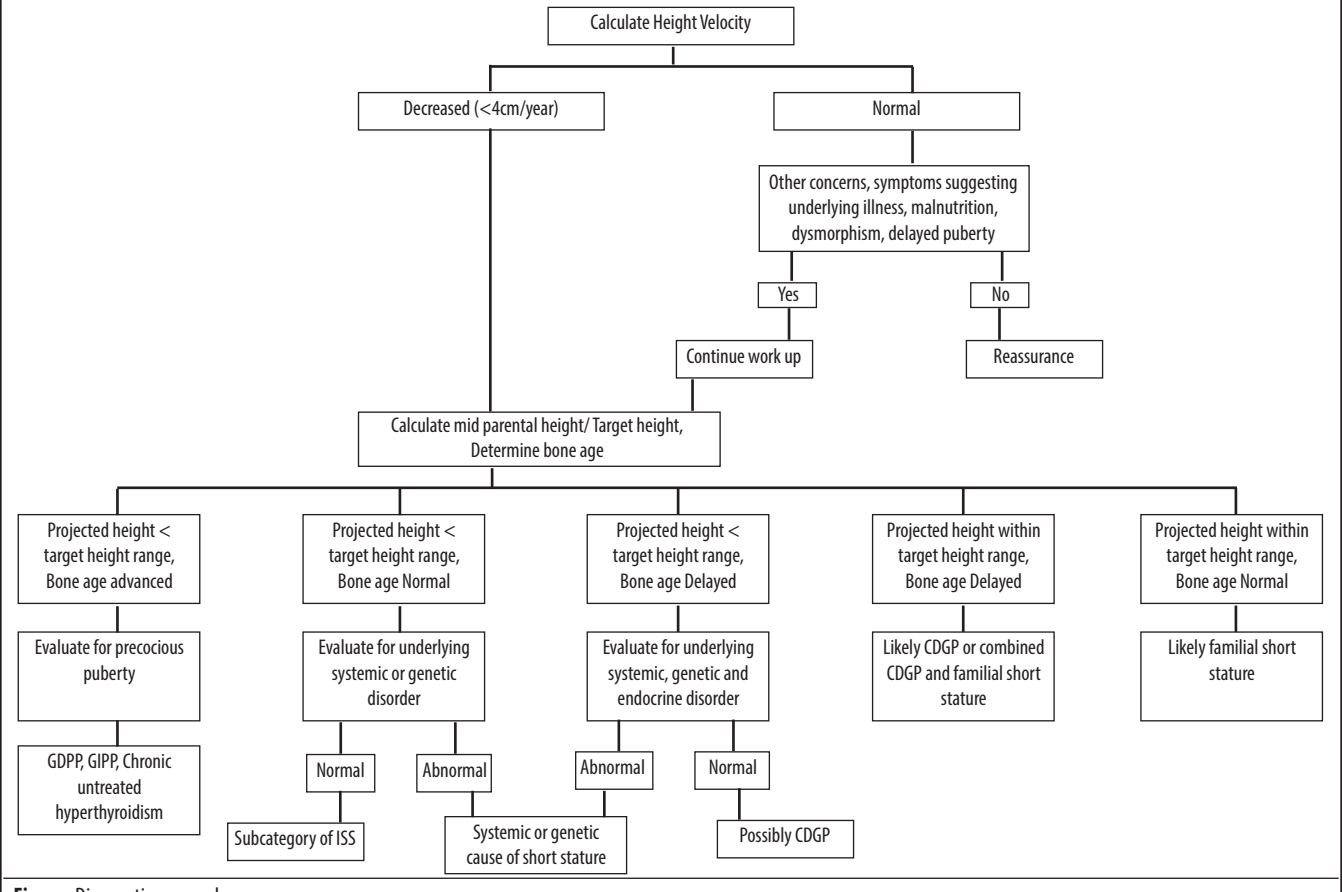

Figure: Diagnostic approach.

GDPP: Gonadotropins-dependent precocious puberty, GIPP: Gonadotropins-independent precocious puberty, CDGP: Constitutional delay in growth and puberty, ISS: Idiopathic short stature.

(CBC), erythrocyte sedimentation rate (ESR), electrolytes, creatinine, alkaline phosphatase (ALP), albumin, calcium, phosphate, thyroid stimulating hormone (TSH), free thyroxine (T4), IGF-I, IGFBP-3 and anti-tissue transglutaminase (tTG) antibodies, were done. Karyotyping was done in girls who had unexplained short stature, and in short boys who also had genital abnormalities. X-ray of the non- dominant hand was done for bone age and was reviewed by an expert radiologist. CBC was done on haematology analyser (Sysmex), for calcium, phosphate, creatinine, ALP and albumin, analyser (Advia 1800, Siemens) was used, for serum TSH, T4, IGF-1, IGFBP-3 and GH, immunoassay analyser (Immulite 2000, Siemens) was used, and anti-tTG antibodies were analysed using enzyme-linked immunosorbent assay (ELISA).

The Centres for Disease Control and Prevention (CDC) growth charts[12] were used to designate height and weight percentiles based on the gender, age, height and weight of the child. The patient's weight and height were determined on a digital weighing system with an attached stadiometer (KERN MPC 250K 100M version 1.3; KERN & Sohan GmbH), with reproducibility of 0.1kg and 0.2cm respectively. To assess GHD, GH levels were assessed after appropriate provocation test. GH response of 20mIU/L or more after stimulation was considered healthy.[13] Priming was done with sex steroids in pre-pubertal children who had bone age >10 years. Prior to the test, ethinyl oestradiol at a dose of 50µg daily was administered to girls for three days, and an injection of testosterone enanthate at a dose of 100mg stat was administered to boys intramuscularly 3-5 days.[14] According to departmental protocol, Levodopa stimulation test was conducted in the morning after 8-10 hours of fasting. Before administering the test, a sample for basal growth hormone analysis was taken. Orally, tablet sinemet 15mg per kg (125/12.5mg tablet for body weight <15kg, 250/25mg tablet for body weight 15-30 kg, and 500/50mg tablet for body weight >30kg) was given. After 1 hour of tablet sinemet administration, a second sample for GH analysis was taken. The exercise stimulation test was carried out in the morning after a fast of 8-10 hours, as per the departmental procedure. Before the exercise, a sample of basal growth hormone was drawn, and then the patient was instructed to run on the treadmill for 20 minutes, and another sample was taken after the exercise for GH analysis. Insulin tolerance test (ITT) was performed in children who had an overnight fast of 8-12 hours, as per departmental protocol. Sample for baseline glucose and GH was taken. At time 0, an intravenous (IV) bolus of insulin at a dose of 0.1IU/kg was given to reduce the plasma glucose levels up to 50mg/dl or half of the basal glucose value. Samples for GH measurement were taken at and after 30 and 60 minutes of hypoglycaemia induction. All children who showed subnormal results after the first provocative test were subjected to another provocative test at least two days later. GHD was diagnosed if the GH peak levels were <20mIU/L. Serum GH was measured using a chemiluminescent immunometric assay (Immulite 2000, Siemens). The method's sensitivity was 0.03mIU/L.

Data was analysed using SPSS 25. Shapiro-Wilk test was applied for assessing data normality. Continuous data was presented as median (interquartile range [IQR]), and categorical data as frequencies and percentages. Chi-square test was used to compare categorical data. Mann Whitney test was applied to compare median levels of IGF-1, IGFBP-3 and peak GH levels in children who were GH-deficient and children who had other aetiologies of short stature. P<0.05 was taken as statistically significant.

Results

Of the 649 children, 422(65.9%) were boys and 227(34.9%) were girls. The overall median age was 11 years (IQR: 5 years) (Table 1). Of the total, 349(53.8%) children were subjected to Levodopa stimulation test, 270(41.6%) underwent exercise stimulation test, and ITT was performed on 30(4.6%) patients.

Various aetiology for short stature status were noted, and 116(17.9%) children were found to have GHD. FSS was present in 130(20%) children and CDGP in 104(16.1%) (Table 2)

There was no significant difference in levels of serum IGF-1 and IGFBP-3 in children who had GHD and those who had other causes of short stature (Table 3).

Table-1: Baseline characteristics (n=649).

Continuous Variables	**Median (IQR)**
Age (years)	11(5)
Height (cm)	116.72 (17.75)
Weight (kg)	21.42(11.89)
BMI (kg/m^2)	15.1(3.85)
Height Velocity (cm)	4.1(0.7)
Mid parental height (cm)	157.76(26)
Bone Age (years)	9.64(1.24)
Peak GH levels (nmol/L)	21(19.5)
IGF-1 (nmol/L)	32(11)
IGFBP-3 (nmol/L)	126(19.58)
Qualitative Variables	**n (%)**
Consanguinity	428(65.9)
Male	422(65.1)
Females	227(34.9)
Preterm	13 (2.0)
LBW(2.5Kg)	56(8.6)

IQR: Interquartile range, BMI: Body mass index, IGF-1: Insulin-like growth factor-1, IGFBP-3: Insulin-like growth factor binding protein-3, LBW: Low birth weight.

Table-2: Aetiology of short stature in study population (n=649).

	Males	Female	Total	*p*-value
	n(%)	n(%)	n(%)	
Physiological Short Stature (ISS)	147(22.7)	87(13.4)	234(36.1)	
Constitutional delay in growth and puberty (CDGP)	63(9.7)	41(6.4)	104(16.1)	>0.05
Familial Short Stature	84(13)	46(7)	130(20)	>0.05
Pathological Short Stature	211(32.5)	204(31.4)	415(63.9)	
1. Endocrine Causes	101(15.6)	82(12.6)	183 (28.2)	>0.05
Growth Hormone Deficiency	72(11.1)	44(6.8)	116(17.9)	>0.05
Hypothyroidism	12(1.8)	14(2.1)	26(3.9)	>0.05
Congenital adrenal hyperplasia	9(1.5)	15(2.3)	24(3.8)	>0.05
Precocious puberty	5(0.7)	5(0.7)	10(1.5)	>0.05
Uncontrolled Diabetes Mellitus	2(0.4)	5(0.7)	7(1.1)	>0.05
2. Systemic Causes	83(12.8)	84(13.0)	167(25.8)	
Anaemia	19(2.9)	51(7.8)	70(10.7)	>0.05
Celiac Disease	33(5.1)	19(2.9)	52(8.0)	>0.05
Asthma and Recurrent Chest Infections	8(1.4)	5(0.7)	13(2.1)	>0.05
Chronic Liver Disease	10(1.6)	8(1.2)	18(2.8)	>0.05
Chronic Kidney Disease	8(1.3)	5(0.7)	13(2.0)	>0.05
Renal Tubular Acidosis	-	1(0.2)	1(0.2)	>0.05
3. Genetic Causes	13(2.0)	14(2.1)	27(4.1)	
Turner Syndrome	-	7(1.1)	7(1.1)	<0.05
Laron Syndrome	13(2.0)	7(1.0)	20(3.0)	>0.05
4. Malnutrition	12(1.9)	21(3.2)	33(5.1)	>0.05
5. IUGR	2(0.3)	3(0.4)	5(0.7)	>0.05

ISS: Idiopathic short stature, IUGR: Intrauterine growth restriction.

Table-3: Comparison of IGF-1, IGFBP-3 and peak growth hormone (GH) levels in children with growth hormone deficiency (GHD) and other causes of short stature.

	Children with GHD Median (IQR) n=116	Children with Other Causes of Short Stature Median (IQR) n=533	*p*- value
IGF-1(nmol/L)	32(11)	32(11)	0.366
IGFBP-3 (nmol/L)	125(19)	126(19)	0.416
Peak GH (nmol/L)	9(5.85)	26.3(13.05)	0.000

IQR: Interquartile range, IGF-1: Insulin-like growth factor-1,
IGFBP-3: Insulin-like growth factor binding protein-3.

Discussion

Endocrinology clinics receive a large number of referrals for the assessment of short stature. In the current study, majority patients were males and were aged 6-10 years. Majority of the studies conducted worldwide have shown the same trend.[15,16]

ISS, which is considered a physiological variant and includes FSS and CDGP, was the most prevalent cause of short stature in the study population. A study reported FSS in 25% of short stature children in Multan, Pakistan.[17] A study in India reported ISS in 66% participants.[18] Only a few short children have some underlying pathology.[19]

In the current study, GHD was present in 116(17.9%) children who presented with short stature. Of them, 68(58.6%) were males. Male-to-female ratio was 2.1:1.5. Takana et al. reported male-to-female ratio of children with GHD 1.59:1.[20] This male predominance does not reflect an actual incidence, but is a result of selection bias as larger number of short stature boys report to clinics for treatment. Parents are more worried about boys' height, and girls are somewhat ignored in this context. GHD is reported in 11.8% short children in Egypt[21] and 9.2% in India.[18]

Malnutrition and anaemia was present in 5.1% and 10.7% of short children in the current study, respectively. A study reported anaemia and stunting concurrently in 9.9% Egyptian primary school children.[22] Anaemia was present in 6.9% children with short stature in India.[18] These easily treatable causes of short stature can be prevented by early diagnosis and appropriate treatment. In the current study, coeliac disease was present in 8% subjects, while it was present in 6.6% in a study in Egypt.[21]

The GH-IGF-1 axis is the fundamental system controlling height in children. GH is the main triggering factor for secretion of IGF-I and IGFBP-3 from the liver into the circulation, and they are decreased in patients of chronic liver disease. However, other than GH, insulin, triiodothyronine (T3), T4 and androgens also stimulate IGF-I release, while oestrogens at low concentration stimulate and inhibit secretion of GH secretion at high concentration. The serum concentration of IGF-I and IGFBP-3 is also decreased in malnutrition and other conditions associated with nutrition, like coeliac disease and anorexia.[23] A single measurement of serum IGF1 and IGFBP3 is more authentic than basal growth hormone levels as IGF-1 and IGFBP-3 have very limited circadian variation. However, the current study did not find any significant difference in serum levels of IGF-1 and IGFBP-3 in children with GHD and with other causes of short stature. Similar results are reported by a study in Japan.[24] Another study also reported that serum IGF-1 levels have poor diagnostic accuracy in differentiating GHD from other causes of short stature.[25]

The current study' limitation is that it was a conducted at a single centre.

Conclusion

GHD was present in 17.9% of short statured children. Isolated GHD, anaemia and coeliac disease were the most prevalent aetiologies involved, but normal variants of development, like FSS and CDPG, as a group were more common. Serum IGF-1 and IGFBP-3 levels alone should not

be used to screen short statured children for GHD.

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Conflict of Interest: None.

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