Original Article

Does priming with sex steroids improve the diagnosis of normal growth hormone secretion in short children?

Ashraf Soliman, Ashraf Adel, Aml Sabt, Elkhansa Elbukhari, Hannah Ahmed, Vincenzo De Sanctis[1]

Department of Pediatrics, Hamad Medical Center, Doha, Qatar, [1]Pediatric and Adolescent Outpatient Clinic, Quisisana Hospital, 44121 Ferrara, Italy

ABSTRACT

Introduction: There is still controversy for priming with sex steroid before growth hormone (GH) testing. **Objective:** We studied GH response to stimulation in 92 children >9 years with idiopathic short stature (height standard deviation score [HtSDS]-2). They were divided randomly into two groups. Children in Group 1 (n = 50) were primed with premarin in girls and testosterone in boys and those in Group 2 were not primed (n = 42). All children were tested using standard clonidine test and their serum insulin-like growth factor-I concentration (IGF-I). Additionally the growth and GH-IGF-I data of the two groups of children were compared with those for 32 short children (HtSDS <–2) below the age 9 years who were non-primed before GH testing (Group 3). **Results:** Neither GH peak response to provocation nor IGF-I concentrations differed between the two groups with and without priming. **Discussion:** Taking a cut-level of 7 ng/ml for normal GH response to clonidine, priming with sex steroids did not significantly increase the percentage of patients with normal GH response (52%) versus nonpriming (47%). IGF-I level did not show any significant difference among the two studied groups >9 years. The peak GH response to clonidine provocation test did not differ before (n = 42) versus after 9 years (n = 32) of age. **Conclusions:** In this randomized study priming with sex steroids before GH testing did not significantly increase the yield of diagnosing short patients with normal GH secretion. In addition, GH response to provocation did not vary significantly between young (<9 years) and old (>9 years) short children.

Key words: Estradiol, growth hormone testing, sex-steroid priming, testosterone

Introduction

The evaluation of a short child begins with a careful medical history and a comprehensive physical examination, including phenotypic characteristics, body proportions and pubertal staging. Calculation of the mid-parental height allows for detecting the genetic background of the child's growth. This is followed by screening laboratory tests, and growth hormone (GH) testing in children with reduced height velocity or low insulin-like growth factor-1 (IGF-1) levels.[1]

Traditionally, the diagnosis of GH deficiency (GHD) results from GH concentrations below an arbitrary cut-off in response to two or more GH stimulation tests (GHSTs).[2] Widely used GHSTs include the insulin tolerance test, arginine, GH releasing hormone, clonidine, and glucagon.[2-4] While provocation tests can diagnose complete GHD, debate still exists about of what constitutes a normal or a subnormal GH response in subjects with "idiopathic" short stature or constitutional delay of growth and puberty. It has been suggested that in children with intermediate GH responses to pharmacologic stimuli (between 7 and 10 ng/ml), a pretreatment with sex steroids priming may be of value in enhancing the GH response and in helping to clarify the diagnosis, particularly in children with delayed onset of puberty.[4]

Access this article online	
Quick Response Code:	Website: www.ijem.in
	DOI: 10.4103/2230-8210.145078

Corresponding Author: Prof. Ashraf T. Soliman, Department of Pediatrics and Endocrinology, Hamad Medical Centre, P. O. Box 3050, Doha, Qatar. E-mail: ATSOLIMAN@yahoo.com

Nevertheless, GH testing in peripubertal children is confounded by the lack of consensus on the use of priming with sex steroids before assessment of the GH-IGF-I axis.[4,5-10] A survey among 235 members of European Society for Pediatric Endocrinology conducted in 2001 showed that 50.2% of the respondents used sex steroid priming in boys and 40.9% in girls.[6]

The aim of our study was to give some contribution to the controversy for priming with sex steroid before GH testing.

Patients and Methods

Ninety-two prepubertal children between the age of 9 and 13 years (58 boys) and 32 children below 9 years were referred to the Pediatric Endocrinology Clinic of Hamad Medical Center (HMC) from June 2010 to June 2013 were evaluated for short stature or growth retardation. They were candidates for testing GH secretion because they had height standard deviation score (HtSDS) <−2 standard deviation (SDs)±, reduced annual growth velocity (growth rate <5 cm/year). None had any systemic or endocrine diseases or history of head trauma or irradiation.

Children >9 years were randomized into two groups: Group 1 received sex steroid priming before performing GHST, and Group 2 did not receive any priming. In boys, intramusculartestosterone depot 25 mg was injected 7–10 days before GH testing; in girls, oral conjugated estrogen 1.25 mg were prescribed for 3 days before testing.[4] Children <9 years did not receive sex steroid priming (Group 3).

A standard oral clonidine ST was performed after overnight fasting at 08:00 am for all the short children.[11] Venous blood was collected at 0, 30, 60, 90 and 120 min for measuring GH concentrations. Serum IGF-I, Free thyroxine, thyroid stimulating hormone and cortisol concentrations were measured at baseline. The maximum GH level achieved during the testing (peak GH) of ≥7 ng/mL was considered as normal. For those with peak GHD, values <7 ng/ml another provocation test with glucagon was performed.[12]

Growth hormone and IGF-I were measured by radio-immunometric assays. Intra-assay coefficient of variation (CVs) was 5.6 and 6.9%, respectively, and inter-assay CVs were 7.9 and 8.2% respectively.

Informed consent was obtained from the subjects, including children over 10 years of age who were old enough to understand the study or their parents. The protocol was approved by the Ethics Committee of HMC, Doha, Qatar.

Statistical analysis

The results for all short children are given as the mean ± SD Anthropometric, and hormonal data were among the three groups using unpaired Student's *t*-test with Bonferroni correction.

Results

The age, bone age and HtSDs of patients in Group 1 (primed before GH testing) did not differ compared to those for group 2 (tested without priming). Peak GH level after stimulation and IGF-I concentrations did not differ among the two groups. Taking a cut-off level of 10 ng/ml for normal GH response to clonidine, priming with sex steroids did not significantly increase the percentage of patients with normal GH response (52%) versus non-priming (47%) [Table 1].

The peak GH response to clonidine provocation did not differ in younger children (Group 3) versus older children >9 years (Groups 1 and 2) of age.

Insulin-like growth factor-I concentrations were correlated significantly with the age of the children ($r = 0.45$, $P < 0.001$) [Figure 1]. No significant correlations between age and basal or peak GH concentrations was observed. HtSDs did not correlate with GH peak or IGF-I concentrations.

Discussion

There is a lack of consensus among clinicians with regards sex steroid priming.[4,13-15] The core argument in favor

Table 1: Anthropometric and laboratory data of short children with (Group 1) and without (Groups 2 and 3) sex steroid priming GH testing

		Age (year)	Bone age (year)	HtSDS	Basal GH (ng/ml)	Peak GH (ng/ml)	IGF-I (ug/L)	Free T4 (pmol/L)
Primed >9 years	Mean	12.0	10.8	−2.3	1.5	11.4	164.1	15.0
Group 1-*n*=50	SD	1.5	1.7	0.34	3.1	6.4	77.6	2.0
Nonprimed >9 years	Mean	12.5	11.1	−2.4	3.0	10.5	160.7	15.3
Group 2-*n*=42	SD	1.4	2.1	−0.3	4.4	7.5	57.9	2.4
Non primed <9 years	Mean	7.2	6.1	−2.2	3.0	10.5	110*	15.3
Group 3-*n*=32	SD	1.6	1.5	−0.4	4.4	7.5	57.9	2.4

Free T4: Free thyroxin 4, SD: Standard deviation, IGF-I: Insulin-like growth factor-I, GH: Growth hormone, HtSDS: Height standard deviation score

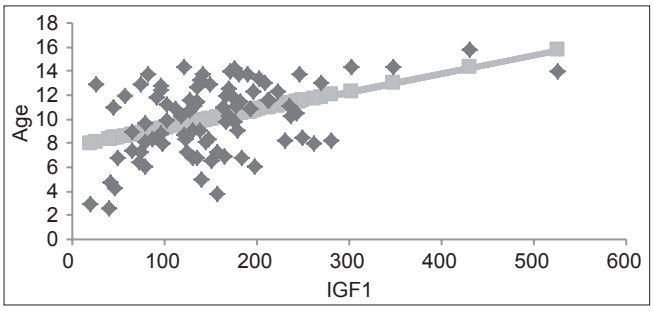

Figure 1: Correlation between age and insulin-like growth factor-I concentrations ($r = 0.45$, $P < 0.001$)

of sex steroid priming during GHST is the improved diagnostic efficiency with the reduction of false-positive GHD diagnoses. On the other hand, the core argument opposed to sex steroid priming targets the possibility of under-diagnosis of true GHD in peripubertal children, as well as the controversial use of GHSTs themselves in the diagnosis of GHD.[4] Some researchers pointed out that both GH provocative testing and sex steroids priming are unphysiologic methods for assessing GH activity in child.[13-15]

In our study, sex-steroid priming did not appear to enhance GHSTs enhances diagnostic efficiency of in prepubertal short children older than 9 years of age because 48% of sex-steroid primed prepubertal children and 53% of non-primed children did not mount normal GH response to GHSTs. Our results are supported by Tillmann *et al*.,[16] who found that the mean GH response to provocative testing did not differ between children who had and who had not undergone priming test. Similarly other authors reported that 61% of normal prepubertal (Tanner stage 1) and 44% of normal Tanner stage 2 children did not reach the GH peak cut-off level (i.e. >7 μg/L [>7 ng/mL]) following the three GHSTs (treadmill exercise, arginine or insulin stimulation).[7]

Contrary to our results, estrogen administration to a subset of prepubertal normal children led to increased GH responses (similar to those observed in Tanner stage 4 and 5 children). They reasoned that estrogen priming in prepubertal (Tanner stage 1) children and in early or mid-puberty (Tanner stages 2 and 3) would be needed to reduce the incidence of false-positive results.[10,15,16]

Other investigators reported improved diagnostic efficiency of GH testing by sex-steroid priming in studies on short children evaluated for possible GHD. Among 23 prepubertal short, normal children, priming with ethinyl estradiol 20 μg/m^2 enhanced the specificity of the levodopa (L-Dopa) GHST for profound GHD.[10,14] Among 39 peripubertal children (Tanner stage 1 or 2) with a mean age of 12 years, and with suspected GHD following an initial GHST with clonidine (priming with 100 mg IM testosterone enanthate for 5–8 days in boys or with 1 mg estradiol valerate daily for 3 days in girls) ruled out GHD in 21 children.[10] The mean peak GH levels increased significantly after priming.[4]

Lazar and Phillip recommend that priming should not be routinely performed in every peri-pubertal child undergoing GH evaluation but may be considered in adolescents with pubertal delay-girls aged >11.5–12 years and boys aged >13–13.5 years exhibiting no evidence of puberty or only initial signs.[13]

Our data support this opinion as in our prepubertal short children (majority below 12 years) sex-steroid priming did not increase the percentage of GH responders compared to those who were not primed nor those younger than 9 years of age.

Conclusion

In this randomized study priming with sex steroids before, GH testing did not significantly increase the yield of diagnosing short patients with normal GH secretion. In addition, GH response to provocation test did not vary significantly between young (<9 years) and old (>9 years) short children.

References

1. Cohen P, Rogol AD, Deal CL, Saenger P, Reiter EO, Ross JL, *et al*. Consensus statement on the diagnosis and treatment of children with idiopathic short stature: A summary of the Growth Hormone Research Society, the Lawson Wilkins Pediatric Endocrine Society, and the European Society for Paediatric Endocrinology Workshop. J Clin Endocrinol Metab 2008;93:4210-7.
2. Pandian R, Nakamoto JM. Rational use of the laboratory for childhood and adult growth hormone deficiency. Clin Lab Med 2004;24:141-74.
3. Rosenbloom AL. Sex hormone priming for growth hormone stimulation testing in pre – And early adolescent children is evidence based. Horm Res Paediatr 2011;75:78-80.
4. De Sanctis V, Soliman AT, Yassin M, Di Maio S. Is priming with sex steroids useful for defining patients who will benefit from GH treatment? Pediatr Endocrinol Rev 2014;11:284-7.
5. Gonc EN, Ozon A, Alikasifoglu A, Kandemir N. Pros of priming in the diagnosis of growth hormone deficiency. J Pediatr Endocrinol Metab 2011;24:9-11.
6. Juul A, Bernasconi S, Clayton PE, Kiess W, DeMuinck-Keizer Schrama S, Drugs and Therapeutics Committee of the European Society for Paediatric Endocrinology (ESPE). European audit of current practice in diagnosis and treatment of childhood growth hormone deficiency. Horm Res 2002;58:233-41.
7. Marin G, Domené HM, Barnes KM, Blackwell BJ, Cassorla FG, Cutler GB Jr. The effects of estrogen priming and puberty on the growth hormone response to standardized treadmill exercise and arginine-insulin in normal girls and boys. J Clin Endocrinol Metab 1994;79:537-41.

8. Martínez AS, Domené HM, Ropelato MG, Jasper HG, Pennisi PA, Escobar ME, *et al*. Estrogen priming effect on growth hormone (GH) provocative test: A useful tool for the diagnosis of GH deficiency. J Clin Endocrinol Metab 2000;85:4168-72.
9. Wyatt DT, Mark D, Slyper A. Survey of growth hormone treatment practices by 251 pediatric endocrinologists. J Clin Endocrinol Metab 1995;80:3292-7.
10. Molina S, Paoli M, Camacho N, Arata-Bellabarba G, Lanes R. Is testosterone and estrogen priming prior to clonidine useful in the evaluation of the growth hormone status of short peripubertal children? J Pediatr Endocrinol Metab 2008;21:257-66.
11. Gil-Ad I, Topper E, Laron Z. Oral clonidine as a growth hormone stimulation test. Lancet 1979;2:1242.
12. Secco A, di Iorgi N, Napoli F, Calandra E, Ghezzi M, Frassinetti C, *et al*. The glucagon test in the diagnosis of growth hormone deficiency in children with short stature younger than 6 years. J Clin Endocrinol Metab 2009;94:4251-7.
13. Lazar L, Phillip M. Is sex hormone priming in peripubertal children prior to growth hormone stimulation tests still appropriate? Horm Res Paediatr 2010;73:299-302.
14. Wetterau LA. The pros and cons of sex steroid priming in growth hormone stimulation testing. J Pediatr Endocrinol Metab 2012;25:1049-55.
15. Moll GW Jr, Rosenfield RL, Fang VS. Administration of low-dose estrogen rapidly and directly stimulates growth hormone production. Am J Dis Child 1986;140:124-7.
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;82:531-5.

Cite this article as: Soliman A, Adel A, Sabt A, Elbukhari E, Ahmed H, De Sanctis V. Does priming with sex steroids improve the diagnosis of normal growth hormone secretion in short children?. Indian J Endocr Metab 2014;18:80-3.

Source of Support: Nil, **Conflict of Interest:** No.