Heliyon 9 (2023) e18713

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Combined growth hormone stimulation testing: Could the tests be shortened?

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ARTICLE INFO

Keywords:
Clonidine
Glucagon
Levodopa
Growth hormone deficiency
Growth hormone stimulation test

ABSTRACT

Introduction: Growth hormone stimulation tests (GHST) remain the cornerstone for diagnosing growth hormone deficiency (GHD), yet they can be lengthy and costly. We aimed to examine whether the combined clonidine and glucagon stimulation test (CGST) and L-dopa and glucagon stimulation test (LDGST) can be shortened without compromising the test's specificity.

Material and methods: We retrospectively analyzed the baseline characteristics, auxological and laboratory data of children with short stature who had undergone a CGST and an LDGST for GHD. We compared the diagnostic accuracy for the standard test and shortened test, eliminating time points of 0 and 210 min.

Results: We reviewed 830 charts (8.17 ± 2.92 years old; 56.27% males), with 431 (57.0%) children in the CGST group, and 38 (51.35%) in the LDGST group who tested negative for GHD. The peak and maximum GH levels occurred at the 60-min time point for both the CGST and LDGST. Eliminating the 0-min time point was the only time that did not affect the specificity of the CGST, with a false-positive rate of 2 (2.99%), specificity of 0.99 (0.99–0.99), and p value of 0.25. Eliminating the 0- and 210-min time points did not affect the specificity of the LDGST, with a false-positive rate of 2 (5.26%), specificity of 0.95 (0.95–0.95), and p value of 0.24.

Conclusions: We concluded that 0-min time point could be eliminated without compromising the combined GHST diagnostic value, thus resulting in cost reduction. Larger studies are needed for the combined LDGST to explore whether the 30- and 210-min time points could be eliminated, thus resulting in cost and time savings.

1. Introduction

Growth hormone deficiency (GHD) is a clinical disorder occurring from the impaired secretion or action of growth hormone (GH). The diagnosis in children is established via a combination of information gathered from the clinical presentation, including auxological measurements, the biochemical analysis of the GH-IGF-1 axis, bone age, and imaging of the hypothalamic pituitary area [1–3]. A gold standard test for confirming the diagnosis of GHD is lacking. The best biochemical proof of impaired GH secretion is assessed by performing growth hormone stimulation tests (GHST) that utilize various provocative agents, including clonidine, arginine, glucagon, L-dopa, growth hormone-releasing hormone and insulin, and obtain serial measurements of GH levels [3–5]. However, GHST have

Abbreviations: Growth hormone deficiency, (GHD); growth hormone, (GH); growth hormone stimulation tests, (GHST); clonidine stimulation test, (CST); glucagon stimulation test, (GST); clonidine-glucagon stimulation test, (CGST); L-dopa-glucagon, (LDGST); Insulin-like growth factor −1, (IGF-1); Insulin-like growth factor-binding protein3, (IGFBP-3).

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https://doi.org/10.1016/j.heliyon.2023.e18713
Received 25 August 2022; Received in revised form 7 July 2023; Accepted 25 July 2023
Available online 26 July 2023

flaws, including the lack of reproducibility and accuracy, the nonphysiological nature of assessments and other practical considerations [6]. Furthermore, a response to a single GHST has a high rate of false positives [4,7]. There is substantial variation in the magnitude of the GH response to various stimuli among the individuals who are tested. Hence, the current consensus mandates the presence of two subnormal GH secretions on provocative GH testing, and it continues to be the diagnostic cornerstone in establishing the diagnosis of GHD [2,4,8]. The repeated GH test results depend on the cutoff point set for the peak GH level, and the diagnosis of GHD can be confirmed or excluded based on the maximum/peak GH level. The standard cutoff peak GH level to exclude the diagnosis of GHD is controversial. However, according to current consensus guidelines, most delegates suggests that inadequate response to two GHSTs with a peak GH level of <7 ng/ml is the cornerstone for the diagnosis of GHD. Yet, adjustments of this threshold should be determined by the pediatric endocrinology society specific to the county [8].

The combined GHST was performed on the same day at the day care center at King Abdulaziz University Hospital (KAUH). Children in the peripubertal period are not primed with sex steroids prior to GHST. There are different GHST protocols and some of the widely used drugs are clonidine and glucagon are the two main stimulant drugs used at KAUH [9]. If the pharmacy does not have clonidine in stock, a 275 mg L-dopa (Sinemet) tablet is used. Clonidine is a selective alpha-receptor agonist that increases growth hormone-releasing hormone (GHRH) secretion and inhibits somatostatin release, thereby increasing GH secretion, and it has been wisely used to test for GHD [3,4,10]. Glucagon is a reliable stimulant drug for evaluating GHD, and it has been proposed that glucagon affects GH release by stimulating alpha adrenergic receptors. In addition, glucagon stimulates GH secretion by rebound hypoglycemia following glucagon-induced hyperglycemia [3,4]. L-dopa stimulates GHRH release via an alpha adrenergic mechanism [5].

There is a lack of consensus on the GHST protocols regarding the duration and peak described in different studies and textbooks. A wide UK survey demonstrated considerable variation in GHST protocol dosing, utilization and interpretation [6]. Furthermore, a Canadian study for pediatric endocrinology practice for growth hormone stimulation tests in various centers showed that the testing duration was not standardized [11]. Therefore, a limited number of previous studies have attempted to elucidate the timing of the GH peak to reduce the total required time and cost of the procedure and found that the usual expected GH peak for the majority of children is at the 60-min time point for the clonidine stimulation test (CST) [11–14], 150-min time point for the glucagon stimulation test (GST) [15,16], and the 30- to 120-min time points for the LDST and that testing could stop earlier without compromising the diagnostic accuracy. Furthermore, Christoforidis et al. [9] concluded that clonidine test blood samples at the 0- and 30-min time points could be omitted without significantly compromising accuracy, while skipping sampling at the 0-, 60-, and 180 min time points for glucagon testing, provided a considerable total false-positive rate of 5.75%.

We hypothesized that the number of GH samples could be reduced without compromising the specificity of the GHST. Shortening the test duration would decrease the burden of the on the children and their families and reduce the cost and time of this dynamic testing. To investigate this hypothesis, we retrospectively reviewed all pediatric patients who had undergone a GHST at KAUH as part of their workup for short stature between January 2014 and January 2021 and examined whether the GHST could be shortened without compromising its diagnostic value.

2. Materials and methods

This was a retrospective cross-sectional study of children who were followed for short stature between 2014 and 2021 at KAUH. Children were included based on the following criteria (1) height below $\leq$ 2 SDs or (3rd percentile), or (2) poor growth velocity below the 25th percentile, or (3) or a decrease in height of at least 0.3 SDs/year, or (4) height that is below the parental target height potential that had undergone two GHSTs using clonidine and glucagon, or L-dopa and glucagon if clonidine was out of stock, to confirm the diagnosis of GHD. The study was approved by the Research Ethics Committee at KAUH. We reviewed the patients' charts and collected the following data: baseline characteristics (age and sex), anthropometric measurements (weight, height, body mass index [BMI]), plotted on the World Health Organization growth charts, Tanner stage according to Marshall and Tanner [17,18], laboratory tests results (Insulin-like growth factor −1 (IGF-1) and Insulin-like growth factor-binding protein3 (IGFBP-3) levels, bone age, GH stimulant drug utilized, and GH as well as glucose levels after combined GHST.

IGF-1 and IGFBP-3 levels were measured by an enzyme-labeled chemiluminescent immunometric quantitative assay (IMMULITE 2000; Siemens Medical Diagnostics, Germany). Bone age was estimated according to Greulich and Pyle method [19], and considered delayed if it alters from the norms for age and gender by at least 1 year.

At KAUH, the standard combined GHST were clonidine-glucagon stimulation test (CGST) or L-dopa-glucagon (LDGST). They were performed after an overnight fast on the same day. A baseline GH level was obtained at the 0-min time point, then clonidine was administered orally at 150 μg/m2 BSA up to a maximum dose of 150 μg and blood samples were taken at the 30- and 60-min time points. If clonidine was not available, L-dopa was instead administered as the first GH stimulant drug at doses of ½ tab, 1 tab, or 1½ tab if the child's weight is < 15 kg, 15–30 kg, and >30 kg, respectively. Glucagon was then administered at the 75-min time point, at a dose of 15 μg/kg IM with a maximum dose of 1 mg, and GH blood samples were obtained every 30 min at the 90-, 120-, 150-, 180-, and 210-min time points. Serum GH levels were quantified using a Siemens Immulite 2000 Systems analyzer two-site chemiluminescent immunometric assay (DPC, Germany). The established GH level peak cutoff value to exclude the diagnosis of GHD was 10 ng/ml at any timed sample. Hence, the diagnosis of GHD was confirmed when the stimulated peak GH concentrations were all <10 ng/ml and was excluded when the stimulated GH concentration was ≥10 ng/ml at any timed sample. The 1st peak GH sample indicated a GH level peak ≥10 ng/ml at only that time point, so its removal would change the interpretation from GH sufficient to GH deficient. The glucose concentrations were measured with the hexokinase-catalyzed glucose oxidase method. Hypoglycemia was defined as a serum glucose level <2.8 mmol/l as defined by the Pediatric Endocrine Society [20].

Our primary outcome was to identify the false-positive (FP) rate of the combined results of the shortened CGST and LDGST without

the 0- and 210-min time points and to compare it to the standard CGST or LDGST including 0 and 210-min time points to diagnose GHD based on a peak GH level <10 ng/ml.

Pediatric patient data were collected using the KAUH electronic data system (phoenix) and then analyzed using the Statistical Package for Social Science (SPSS) version 22.0 for Mac to determine the mean and standard deviations or the median and 25th and 75th percentiles, depending on the distribution of data. Categorical data are displayed as frequencies and proportions. We determined the difference between the proportion of children who tested positive for growth hormone deficiency on the standard duration tests with children who tested positive on the new shortened duration tests using McNemar tests (for paired proportions). We then reported the frequency of sufficient peak GH levels ≥10 ng/ml and the first time GH level ≥10 ng/ml in those who tested negative for GHD after the combined CGST and LDGST. A p value ≤ 0.05 was chosen as the level for statistical significance.

3. Results

A total of 830 GHST were performed to evaluate short stature children for GHD. Table 1 displays the baseline characteristics of the short stature children who had undergone a combined GHST with both the CGST and LDGST. The mean age was 8.17 ± 2.92 years; 56.2% were males, IGF-1 SDs was −0.94 ± 1.29, and 40% had delayed bone age. Table 2 demonstrates the peak and maximum GH stimulation levels among the children who tested negative for GHD (GH level ≥10 ng/ml). There were 765 children who underwent a CGST, the prevalence of GHD was 431 (57%), the majority (302; 39.95%) peaked at the 60-min time point, followed by 190 (25.13%) who peaked at the 90-min time point. The majority (255; 33.73%) of the children also had their 1st GH level above 10 ng/ml at the 60-min time point, and 49 (6.48%) at the 90-min time point. A total of 74 children underwent LDGST with a GHD prevalence of 38 (51.35%), the majority (19; 25.68%) peaked at the 60-min time point, followed by 13 (17.57%) children who peaked equally at the 120- and 150-min time points. The majority (16; 21.62%) also had their 1st GH level ≥10 ng/ml at the 60-min time point, and 6 (8.1%) at the 90-min time point. Of interest, only one child (1.35%) peaked at the 30-min time point. There was no association between hypoglycemia and the time of the peak, the peak among patients with hypoglycemia occurred at 60 min prior to glucagon administration.

Table 3 shows the diagnostic accuracy of the shortened GH stimulation test with clonidine and glucagon. Among the 756 children tested, 431 (57%) tested negative for GHD with a peak ≥10 ng/ml. Eliminating the 0-min time point was the only time that did not affect the specificity of the CGST, with a false-positive rate of 2 (2.99%), specificity of 0.99 (0.99–0.99), and p value of 0.25.

Table 4 shows the diagnostic accuracy of the shortened GH stimulation test with L-dopa and glucagon. Among the 74 children tested, 38 (51.35%) did not have a peak ≥10 ng/ml, diagnosing them with GHD. Eliminating the 0- and 210-min time points did not affect the specificity of the LDCST, with a false-positive rate of 2 (5.26%), specificity of 0.95 (0.95–0.95), and p value of 0.24.

4. Discussion

Our study explored the diagnostic accuracy of the shortened combined CGST or LDGST performed with a large cohort of 813 children. Our data indicated that eliminating the 0-min samples from the CGST did not alter the interpretation of GH testing based on a GH cutoff of 10 ng/ml with an acceptable false-positive rate of 2 (2%). Similar to our study, Christoforidis et al. [9] demonstrated that the 0-min time point could be omitted without significantly compromising the diagnostic validity of the GHST. Gillis et al. [14]

Table 1
Baseline characteristics.

	Total cohort N = 830	Clonidine + Glucagon N = 756	L-Dopa + Glucagon N = 74
Age, years	8.17 ± 2.92	8.19 ± 2.95	7.94 ± 2.63
Sex, male *	467 (56.27)	440 (58.20)	27 (36.49)
Height, cm	113.87 ± 16.97	113.96 ± 17.12	113.0 ± 16.58
Height, SDs	−2.27 ± 1.23	−2.27 ± 1.23	−2.29 ± 1.27
Weight, kg	21.7 (10.62)	21.91 ± 10.88	19.61 ± 7.03
Weight, SDs	0.28 ± 1.44	−1.82 ± 1.44	−2.10 ± 1.31
BMI, kg/m^2	15.66 ± 3.31	15.74 ± 3.3	14.92 ± 2.1
BMI, percentile	33.20 ± 31.65	33.75 ± 31.99	27.6 ± 27.6
Tanner stage *			
I	577 (69.77)	522 (69.23)	55 (75.34)
II	169 (20.41)	152 (20.13)	17 (23.29)
III	62 (7.5)	61 (8.09)	1 (1.37)
IV	18 (2.18)	18 (2.39)	–
V	1 (0.12)	1 (0.13)	–
IGF-1 (ng/mL)	110 ± 2.42	111.04 ± 69.21	103.45 ± 60.66
IGF-1, SDs	−0.94 ± 1.29	−0.93 ± 1.30	−1.05 ± 1.9
IGFBP-3 level (ng/mL)	3411.52 ± 1237	3432 ± 1250	3189.55 ± 1071
Bone Age, years	6.76 ± 2.99	6.76 ± 3.03	6.86 ± 2.62
Delayed bone age *	329 (39.6)	303 (40.0)	26 (35.1)

All values are the mean ± standard deviations (SDs) unless indicated.
*N (%).
BMI, body mass index; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor-binding protein 3.

Table 2
Peak and maximum GH stimulated levels among children who tested negative for GHD.

	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Clonidine + Glucagon N = 756 GH maximal response N (%)	12 (1.59)	52 (6.88)	302 (39.95)	190 (25.13)	137 (18.12)	121 (16.01)	76 (10.05)	31 (4.10)
GH 1st level ≥10 N (%)	12 (1.59)	49 (6.48)	255 (33.73)	49 (6.48)	25 (3.31)	18 (2.38)	16 (2.12)	7 (0.93)
L-Dopa + Glucagon N = 74								
GH maximal response N (%)	4 (5.41)	1 (1.35)	19 (25.68)	11 (14.86)	13 (17.57)	13 (17.57)	8 (10.81)	3 (4.05)
GH 1st level ≥10 N (%)	4 (5.41)	–	16 (21.62)	6 (8.11)	5 (6.76)	3 (4.05)	3 (4.05)	1 (1.35)

Table 3
Specificity of the shortened GH stimulation test with Clonidine and Glucagon. (N = 756).

Test	True positive N (%)	True Negative N (%)	False-positive N (%)	Specificity (95th CI)	P value	+ LR[a]
Without the 0-min time point	429 (56.75)	325 (42.99)	2 (0.26)	0.99 (0.99–0.99)	0.25	215.5
Without the 210-min time point	424 (57.08)	325 (42.99)	7 (0.93)	0.98 (0.98–0.98)	0.007	61.6
Without the 0- and 210-min time points	421 (55.69)	325 (42.99)	10 (1.32)	0.98 (0.98–0.98)	<0.001	43.1

[a] Likelihood ratio.

Table 4
Specificity of the shortened GH stimulation test with L-dopa and Glucagon. (N = 74).

Test	True positive N (%)	True Negative N (%)	False-positive N (%)	Specificity (95th CI)	P value	+ LR[a]
Without the 0-min time point	38 (51.35)	36 (48.65)	0 (0)	1.00	1	Infinity
Without the 210-min time point	37 (50)	36 (48.65)	1 (1.35)	0.97 (0.97–0.97)	0.5	38
Without the 0- and 210-min time points	36 (48.65)	36 (48.65)	2 (2.70)	0.95 (0.95–0.95)	0.24	19

[a] Likelihood ratio.

reported that removal of the 0-min samples would have altered the results of 1.2% of the CSTs. In our institution, a single GH analysis test costs 180 SAR (~50 USD); thus, eliminating the 0-min time point would lead to notable financial savings of ~150,000 SAR (~40,000 USD), which could have been allocated to alternative health care services.

Our data confirm the observations of other studies that showed that the 60-min time point was the highest peak and had the max stimulated GH levels after CST [9,11,14,21].

The same day combined GH stimulation test protocol utilized in our center is different than that utilized in many centers, so it would be difficult to compare the glucagon peak level in our study to that of other studies. Interestingly, after glucagon administration, we noticed a lower-than-expected 1st GH level ≥10 ng/ml at all time points when compared to clonidine administration (15.22% vs. 33.73%). In contrast to our findings, the reported percentages of 1st GH levels ≥10 ng/ml (negative for GHD) were 35.6% and 54.02%, respectively, after glucagon tests in 2 other studies [9,15]. A further study by Secco et al. [22] found that a GH peak to glucagon >10 ng/ml occurred in 58.3% of their cohort of children. The lower response to glucagon observed in our study might mainly be because only 103 (12.39%) of all children developed hypoglycemia during combined GHST, which is the main mechanism that stimulates GH release under the influence of the glucagon hormone. Interestingly all the combined GHST false positive cases did not develop hypoglycemia during the test, so eliminating those children lead to a false positive rate of zero.

Our study indicates that omitting the 0- and 120-min time points would not affect the specificity of the test in diagnosing GHD, with an acceptable FP rate of 2 (5.26%). Only one child had a peak GH level ≥10 ng/ml at 30 min, and no children peaked ≥10 ng/ml at 30 min alone, which indicates that this time point could be omitted from GHST as well. The accuracy of a single LDST in positively predicting GHD ranges between 81% (cutoff = 6 mcg/l) and 56% (cutoff = 7 mcg/l) [5].

A major limitation of our study is the diagnostic accuracy and poor reproducibility of the combined GHST in diagnosing GHD. However, we used the same stimulant drugs and an identical protocol, and the GH levels were measured in the same lab utilizing one reliable assay. The use of the combined GHST has been described in the literature [5,23,24], yet there have been no studies documenting the use of both the CGST and LDGST, like our protocol. In addition, due to the selection criteria of the study population, some children were tested with a low pretest probability for GHD, specially that we did not correlate the GHST results with the clinical indices of GH status, such as growth velocity and brain MRI findings. Furthermore, the findings for the children who had undergone an LDGST are of limited significance given the small sample size. However, this was expected since L-dopa was the second drug of choice because clonidine was out of stock.

5. Conclusions

In conclusion, this study showed that the peak and maximum GH levels occurred at the 60-min time point for both the CGST and

LDGST. It also determined that the 0-min time point could be eliminated without compromising the combined GHST diagnostic value, hence leading to financial savings. Larger studies are needed for the combined LDGST to explore whether the 30 and 210-min time points could be eliminated, thus resulting in cost reduction, time savings, and more convenient fasting duration for children and their families.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethics statement

- This study was approved by the National Committee of Bio & Med ethics - King Abdul Aziz University, with approval number: [*236-21]*.
- Informed consent was not required for this study because it was a retrospective cross-sectional study that involved no risk to subjects and no breach of patient's confidentiality.

Author contribution statement

Tarah H Fatani, MD, FAAP, FRCPC: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

We would like to thank Dr. Reem Al- Khalifah for the scholarly discussion and data analysis that contributed to developing this article.

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