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The arginine stimulation test: timing of peak is not a helpful parameter in the diagnosis of growth hormone deficiency

Abstract

Background: A typical peak timing in the glucagon stimulation test has been reported as an indication of growth hormone (GH) deficiency. Other stimulation tests have not been evaluated.

Objective: To evaluate the clinical usefulness of peak timing in the arginine stimulation test (AST) for growth hormone deficiency.

Methods: Retrospective review of 199 ASTs from one center. Outcomes included correlation of peak times with (a) frequency of deficient peak; (b) growth velocity standard deviation scores (GVSDSs); (c) other evidence of pituitary pathology; (d) results of confirmatory clonidine test; and (e) response to GH treatment.

Results: The peak in 83/109 (76.14%) sufficient tests occurred at typical times vs. 45/72 (62.5%) deficient tests ($p<0.05$). GVSDS on GH treatment was greater among patients with typical timing in the AST compared with atypical timing (2.67±0.59 vs. 0.46±1.17, $p=0.021$). No other variable correlated significantly with AST timing.

Conclusions: Timing of peak in the AST is not a clinically useful parameter.

Keywords: arginine; growth hormone deficiency; short stature.

[a]David Gillis and Nadav Granat were equal contributors to this study. Nadav Granat's participation in this study was performed in fulfillment of the research requirements towards the MD degree at the Hebrew University Hadassah School of Medicine.

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Introduction

The arginine stimulation test (AST) is a commonly used dynamic test for the evaluation of growth hormone (GH) deficiency (1). In this test, blood samples are taken before intravenous administration of arginine and then at further fixed times over the next 2 h. Interpretation of this test relies solely on the maximum (peak) value of the samples; currently, in Israel, a peak above 7.5 ng/mL according to the current standard, or 10 ng/mL according to the old standard (for details, see the Methods section), is considered indicative of GH deficiency (2). In our practice, as previously reported, the most common time for occurrence of the peak value was at 45 min after injection (3). This was similar to the peak times reported in the earliest descriptions of this test (4, 5). Our group reported previously that, for the glucagon stimulation test (GST), most patients had peak levels of growth hormone 90 or 120 min after stimulation. Those patients whose peak growth hormone levels occurred either unusually early after stimulation (i.e., before 90 min) or unusually late (i.e., more than 120 min after the stimulus) were more likely to have other evidence for GH deficiency. We termed these as "atypical" timing compared with tests at which peak occurred 90 or 120 min after glucagon administration which were termed "typical" (6). We suggested that similar studies should be undertaken for other growth hormone stimulation tests in order to evaluate the clinical usefulness of timing as a parameter of growth hormone secretory dynamics. We therefore retrospectively analyzed all ASTs performed in a single center and ordered by a single physician (DG) between October 2007 and September 2011.

Methods

AST is the first dynamic test performed at the Hadassah-Hebrew University Medical Center when GH deficiency is suspected. Indications for evaluation of GH status vary, but generally include height less than the third percentile or height standard deviation score significantly lower than family appropriate target range, an abnormal growth rate and a delayed bone age. Testing for GH deficiency is

undertaken after exclusion of other causes of growth retardation such as anemia, celiac disease, hypothyroidism, renal or liver disease, and chronic inflammation (6).

The AST procedure is performed as follows: a blood sample (designated 0 min) for growth hormone is taken prior to intravenous infusion of arginine (0.5 g/kg body weight administered over 30 min). The next sample is taken immediately after the infusion (designated the 30-min sample) and then at 45, 60, 90 and 120 min. Girls aged 11 years and 6 months or above are primed with 2 mg of beta estradiol (or equivalent estrogen) orally each day for 3 days up to the day before the test. Boys aged above 13 years are given a single intramuscular injection of 100 mg testosterone enanthate 7–10 days prior to the test. Currently, serum growth hormone concentration is tested using the Immulite 2000 analyzer chemiluminescent test (Siemens, Erlangen, Germany) with the international standard IS 98/574 (7). The current standard was introduced in our laboratory on July 1, 2010, and therefore the results of GH tested before July 2010 were normalized according to the new figures, i.e., multiplied by 0.75.

As mentioned above, in Israel the AST is considered indicative of GH deficiency if the highest GH level is less than 10 ng/mL using the older standard or 7.5 ng/mL with the current IS 98/574 standard. When the AST peak is below these thresholds, a second confirmatory test, with clonidine as the GH stimulant, is performed. If the peak in the clonidine stimulation test (CST) is also below 7.5 ng/mL, GH deficiency is diagnosed. When there is a priori radiological evidence of a condition associated with GH deficiency (e.g., ectopic posterior pituitary or "empty sella") or if the patient has another pituitary hormone deficiency, then a single deficient stimulation test (usually the AST) is considered diagnostic. Results of all ASTs performed in our center and ordered between October 2007 and September 2010 by a single physician (DG) were reviewed. In total, there were 199 ASTs. Five patients underwent two ASTs during this time and we arbitrarily analyzed only the latter of the two in order to avoid intra-patient variation. For 13 tests, the peak occurred before stimulation. These were excluded from the current analysis since the peak time was thought to have reflected the fluke occurrence of a spontaneous GH peak just prior to the arginine stimulus. Since there is a refractory period immediately after a burst of growth hormone secretion during which the pituitary's response to stimuli is reduced (8), these tests could not be included when evaluating the timing of response to arginine. After the exclusions, 181/199 tests were analyzed further in order to evaluate the significance of the peak time.

Growth velocity was based on height measurements taken during clinic visits between 3 and 15 months prior to the test. Growth velocity standard deviation scores (GVSDSs) were calculated using the Auxology program version 1.0 (Copyright 2003 Pfizer Pharmaceuticals) according to the UK-Tanner reference tables 2000.

Evidence for pituitary pathology was defined as presence of one or more of the following: at least one other pituitary hormone deficiency, familial GH deficiency, an anatomic anomaly of the pituitary known to be associated with GH deficiency based on magnetic resonance imaging or history of irradiation. These data were collected from electronic clinic notes of one of us (DG).

Statistics

In order to investigate the correlation between two qualitative variables, we used the chi-square test or the Fisher's exact test. In order to compare two independent groups for quantitative variables, we used either the t-test or a parametric Mann-Whitney test. To investigate changes within a group for quantitative variables, we used the matched t test or a parametric Wilcoxon test.

The data collected included birth date, growth data, parental heights, arginine test results, clonidine test results and details of past medical history. Results are presented as average±standard error.

Ethics

The chart review was approved by the institutional review board of the Hadassah-Hebrew University Medical Center.

Results

A total of 199 arginine tests were performed, of which before exclusions 81 were considered deficient and 118 were considered sufficient. Average results for each sample time for tests defined as deficient vs. results for tests defined as sufficient are presented in Table 1. After applying exclusion criteria (see Methods), 181 arginine tests were included in the analyses regarding the importance of peak times. Among these, 45 min was the most frequent peak time (in 31.5% of the tests) (Figure 1). A total of 109 (60.2%) of 181 test results indicated GH sufficiency, i.e., peak GH was over 7.5 ng/mL (or 10 ng/mL before July 1, 2010) in at least one sample.

We defined early peak times as "typical" since, of the total five time points studied, the observed frequency of the peak occurring at the three earliest time points (30, 45 and 60 min) was 70.7%, significantly greater than the expected 3/5=60% ($p<0.005$). Conversely, peaks occurring at the two later times (i.e., 90 or 120 min) were defined as "atypical". Of note, among 109 tests defined as "sufficient", the average serum GH was above the threshold of 7.5 ng/mL at each of the three sample times designated as "typical" (Table 1). The average age

Table 1 GH results for each time sample for 81 deficient tests and 118 sufficient tests.

Deficient tests, ng/mL	Sufficient tests, ng/mL	Sample time, min
mean±SE	mean±SE	
1.61±0.2	2.74±0.4	0
3.31±0.27	10.58±0.9	30
3.4±0.29	12.56±1.0	45
3.05±0.29	10.86±0.9	60
2.57±0.25	7.43±0.7	90
2.84±0.28	6.48±0.7	120

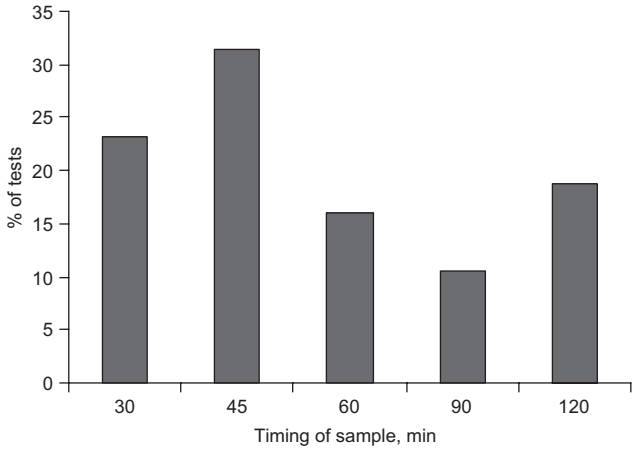

Figure 1 Histogram depicting the distribution of peak times among 181 samples.

at testing among the 181 tests was 8.9±3.7 years (range 0.65–17.46). The two groups (typical vs. atypical) were comparable in terms of proportion of the group primed with sex hormones (typical: 25/128=19.5%, atypical: 8/53=15.1%; p=0.48).

Frequency of typical times vs. test result in the arginine test

The peak GH levels in tests indicating GH sufficiency ("sufficient tests") occurred more often at typical times when compared to tests indicating GH deficiency ("deficient tests"). Among the sufficient tests, 83/109 (76.14%) occurred at typical times. Among deficient tests, 45/72 (62.5%) occurred at typical times (χ^2=3.9, p<0.05).

Consistency of typical/atypical timing upon repeated testing

Of the five patients tested twice, three patients were consistent vis-à-vis typicality (two remained typical and one remained atypical upon repeat testing). Two were inconsistent: one turned from atypical to typical but was prepubertal at the first test and pubertal at the second test 4 years later which could perhaps explain the difference. The other one who turned from typical to atypical was tested 2 years apart, and, actually, both tests were indicative of growth hormone deficiency, so that the difference may indicate a deterioration in GH secretory status whereby initially the child was deficient but with typical timing and later was deficient with atypical timing. Thus, taken together, the repeated tests do not contradict the idea of peak timing as a significant factor in GH secretory status.

Growth velocity of patients with deficient ASTs

For 60 of the 72 deficient tests, GVSDS was available. The AST among 35 of these was typical. Average GVSDS was lower for the 25 children whose AST was atypical but did not reach statistical significance (–1.75±0.45 vs. –0.92±0.42, p=0.19).

Growth velocity of patients with sufficient ASTs

The GVSDS of 88/109 patients with a sufficient AST result was available. The AST of 70/88 was typical. The average GVSDS was significantly higher for the 18 children whose AST was sufficient and atypical compared with sufficient and typical (0.2±0.75 vs. –1.88±0.26, p<0.01).

Atypical timing of peak and results of confirmatory test

A total of 63 of 72 deficient ASTs were followed by a confirmatory clonidine stimulation test (CST). Of these ASTs, 39/63 peaked at typical timing and 24/63 were atypical. Seventeen (43.6%) of 39 patients with typical deficient ASTs were confirmed GH deficient by the CST and 10 (41.7%) of /24 atypical ASTs were confirmed as GH deficient (p=n.s.). There was therefore no significant correlation between typicality of timing in the AST and final result in terms of deficiency or sufficiency of the confirmatory CST.

Pituitary pathology and timing of peak

Among our cohort, 27 patients had one or more of the following: multiple pituitary hormone deficiency, familial GH deficiency, history of radiation for a brain tumor, and anatomic anomaly of the pituitary based on magnetic resonance imaging. Such evidence of significant pituitary pathology was noted nominally more frequently among patients with atypical ASTs (11/53, 20.8%) compared with typical ASTs (16/128, 12.5%) (p=0.24, Fisher's exact test, two tailed).

Growth velocity on growth hormone treatment

We compared the growth velocity of patients who were treated with growth hormone for at least 6 months. Among 16/22 treated patients with available data, the peak time of the AST was typical. Average GVSDS was significantly higher for those patients compared with the 6/22 patients who, prior to therapy, had atypical ASTs (2.67±0.59 vs. 0.46±1.17, p=0.021).

Discussion

Currently, the sole parameter considered by clinicians interpreting the AST is the peak GH level at whatever time it occurs. The aim of this study was to evaluate whether, as for the GST (6), the time at which the peak occurs could be another useful parameter. The results of the present study did not define a clinically useful role for timing of the peak in the diagnosis of growth hormone deficiency by the AST. In order to reach this conclusion, the first step in our analysis was to characterize typical timing. We defined this based on those times that occurred more frequently than expected. Furthermore, in the current study, for ASTs considered sufficient, the average serum growth hormone was above the standard 7.5 ng/mL threshold at, and only at, each of the three times that were determined typical. These times are, in our opinion, valid for the above reasons and also because they are in agreement with the typical peak times determined in several previous studies (3–5).

The findings of the present study regarding the lack of utility of the peak time as a useful parameter contrast with those of our previous study of the GST (6). In that study, among those whose peak GH level was indicative of GH deficiency, atypical timing of the peak increased the chance that deficient secretion would be confirmed in a clonidine stimulation test (6). The other major finding in that study was that patients with deficient atypical GST results and sufficient confirmatory tests had a lower GVSDS than patients with deficient typical results and sufficient confirmatory tests, although data regarding this issue were available only for a small number of patients. There was also an excellent correlation between "typicality" of the timing in deficient GSTs and typicality of timing in the confirmatory clonidine test (6). The reasons for the different findings in the present study are unclear. Indeed, the total percentage of typical ASTs (70.7%) in the current study was similar to the percent of typical GSTs (76%) and the highest average level of 12.56±1.0 ng/mL for the AST (at 45 min) was similar to the 11.2±0.5 ng/mL for the GST (at 120 min) (6). Also, physiologically, disordered patterns of secretion have been described as part of the growth hormone deficiency syndrome (9, 10). Therefore, we expected to find that timing in the AST would be of similar importance and potential usefulness. Indeed, the peak in ASTs indicating GH sufficiency tended to occur more often at typical times (30, 45 and 60 min) compared with the peak in tests indicating insufficiency. This can be interpreted to mean that children who, in response to arginine, secrete GH in adequate quantity tend to do so also rapidly, which would indicate physiological importance to timing. Also, children with evidence of pituitary pathology other than stimulation test results have a nominally higher chance of atypical timing in the AST, similar to the statistically significant trend for the GST. However, the results for correlation between timing of the peak and growth rates were dependent on the level of the peak. Among children whose AST peak was high, i.e., indicative of sufficiency, typical timing was associated with lower growth velocity. Contrarily, among those whose AST peak was low, i.e., indicative of deficiency, typical timing was not associated with higher growth velocity and, in fact, typical timing was nominally associated with lower velocity, although data regarding this issue were available only for a relatively small subgroup of patients. We do not have a reasonable explanation for this finding, but whatever its cause, it means that peak timing is not an independent parameter since the effect of timing on growth varies depending on peak level. Lastly, the growth response was contrary to what would have been intuitively expected – there was a significantly decreased growth response to treatment among patients whose arginine peaks occurred at atypical times. This may reflect a concomitant inability to secrete factors downstream to GH among some of these children whose GH secretion is deficient, which could occur, for example, if there was a general disorder of exocystosis (11). More likely, however, this simply reflects the known limited correlation between GH stimulation test results and response to therapy (12).

Overall, we conclude that, although atypical peak GH responses may indeed be a physiological indication of poor GH secretion, the effect does not appear to be potentially useful in the clinical definition of GH deficiency by the AST.

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