

Growth hormone (GH) peak after falling asleep reflects spontaneous nocturnal GH secretion, however is not corresponding to the results of GH stimulating tests in children with short stature

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Abstract

OBJECTIVE: Growth hormone (GH) secretion is characterized by a pulsatile, circadian rhythm, with the highest concentrations at night hours. Evaluation of nocturnal GH secretion may be truncated to 6 hours. Growth hormone stimulating tests are the standard method of assessment of GH secretion. In Poland, the assessment of GH peak during 2 hours after falling asleep was introduced as a screening procedure in children, suspected for GH deficiency.

The aim of current study was to compare the results of a screening test with GH secretion during 6-hour nocturnal profile and with the results of GH stimulating tests, as well as with IGF-I secretion in children with short stature.

METHODS: In 72 short children, GH concentrations were measured every 30 minutes during first 6 hours after falling asleep and in two GH stimulating tests (the cut-off level of GH peak for all the tests was 10.0 ng/ml). Also, IGF-I concentrations were measured and expressed as IGF-I SDS for age and sex.

RESULTS: The screening test results correlated significantly with both GH peak in 6-hour profile and mean GH concentration, and the area under the curve (AUC) in 6 hour profile ($r=0.94$, $r=0.90$ and $r=0.89$, respectively, $p<0.05$) but not with GH peak in stimulating tests ($r=0.07$, NS). There was no correlation between IGF-I secretion and any of the analyzed parameters of spontaneous and stimulated GH secretion.

CONCLUSIONS: The results of screening test seem to reflect overnight GH secretion in short children, remaining, however, discordant with the results of GH stimulating tests and with IGF-I secretion.

Abbreviations:

AUC - area under the curve
CV - coefficient of variation
GH - growth hormone

GHD - growth hormone deficiency
IGF-I - insulin-like growth factor-I
rhGH - recombinant human growth hormone
SDS - standard deviation score

INTRODUCTION

Growth hormone (GH) secretion is characterized by a circadian pattern, with the highest concentrations after falling asleep. Moreover, three different rhythms of GH secretion were identified: pulsatile (controlled by GH releasing hormone and somatostatin), entropic (related to different intra- and extrapituitary signals converging on somatotroph cells) and nycthemeral (24-hour) (Shah *et al.* 1999; Veldhuis & Bowers 2003). As GH is secreted in a pulsatile manner, the increases in GH serum concentration (peaks) may be detected only by serial sampling in 20–30 minute intervals. Thus, the exact assessment of spontaneous GH secretion is quite onerous, both for the patient and for the medical staff (who should not disturb patients' sleep and spontaneous activity), and requires sophisticated methods of analysis. The study of Albertsson-Wikland *et al.* (1994) proved that in healthy children both GH secretion rates and the number of peaks were sex-specific and related to the stage of puberty. In other study, Saggese *et al.* (1993) found that in short children nocturnal, 12-hour GH secretion was a reliable index of GH secretory status. Then, Rose and Municchi (1999) demonstrated that overnight 12-hour evaluation of GH secretion may be truncated to 6 hours (from 22:00 to 04:00). However, according to the Consensus Guidelines of GH Research Society (2000), the evaluation of spontaneous GH secretion (12- or 24-hour) can be considered only if decreased insulin-like growth I (IGF-I) remains in discordance with normal results of GH stimulating tests, *i.e.* for the diagnostics of neurosecretory dysfunction (NSD). Ten years later, Webb and Dattani (2010) have paid an attention to the fact that quantifying of overnight GH release may not identify all the subjects with GH deficiency (GHD), due to the intraindividual variation of spontaneous GH secretion, as it was reported 15 years earlier by Rosenfeld *et al.* (1995). The lack of normative data, pertaining to age, sex and body mass index, as well as the time- and labour-intensiveness of that test were also stressed in the quoted study. Thus, so far, GH stimulating tests are recommended as the most widely used and accepted tools in diagnosing GHD, as it has very recently been confirmed by Webb and Dattani (2010).

In Poland, the assessment of GH peak during 2 hours after falling asleep was introduced a few years ago as a screening procedure in short children, suspected of GHD, with the cut-off value of GH peak on the same level as for stimulating tests (*i.e.* 10 ng/ml). According to the rules of interpreting the screening test results, it is sufficient to subject to GH stimulating tests only those patients, who demonstrated decreased GH peak in screening test. On the other hand, GH stimulating tests are necessary to confirm the diagnosis of GHD. These recommendations are in contradiction with the statement of GH Research Society (2000), corroborated by the recommendations of other authors (Webb &

Dattani 2010). At the same time, Polish recommendations are consistent with other findings, for example with these presented by Radetti *et al.* (2003), who have stressed the effectiveness of recombinant human GH (rhGH) therapy only in the patients with decreased spontaneous GH secretion (independently from the results of stimulating tests). So far, only scarce data are reported indicating that GH peak in screening test well reflects nocturnal GH secretion. The results of our previous study (Smyczynska *et al.* 2010) indicated that the highest GH peak after falling asleep was observed during the first 2 hours of 6-hour test, while only in 4% of the studied patients the peak in question occurred later. However, that study was conducted in order to assess the reproducibility of nocturnal GH secretion, so other parameters of GH rhythm were not analyzed.

The aim of present study has been to compare the results of screening test (GH peak during 2 hours after falling asleep) with GH secretion during 6 hours of nocturnal profile and with the results of GH stimulating tests, as well as with IGF-I secretion in children with short stature.

PATIENTS AND METHODS

The analysis comprised 72 children (47 boys, 25 girls), age 11.2 ± 2.9 (mean \pm SD) with short stature (*i.e.* patients' height below 3rd centile for age and sex), delayed bone age and a slow growth rate (below 4 cm/year). In each child, nocturnal GH secretion was assessed in 6 hour profile (including 11 samples every 30 minutes – starting from 1 hour after falling asleep) and two standard GH stimulating tests (with clonidine 0.15 mg/m², orally and with glucagon 30 μ g/kg, *i.m.*, not exceeding 1.0 mg) were performed, with the cut-off value for normal and decreased GH peak on the level of 10.0 ng/ml, established arbitrarily, according to Polish recommendations. Simultaneously, IGF-I secretion was assessed; fasting blood samples were collected in morning hours, the day after nocturnal GH profile. All the children with either any chronic diseases that may affect GH secretion and action, or genetic syndromes, or with acquired causes of growth failure, were excluded from the study.

The authors feel to be obliged to admit that, as 6-hour GH secretion profile is not an obligatory procedure, a standard diagnostics towards GHD was executed in all the patients earlier, and the assessment of 6 hour profile was performed only if the results of 2-hour screening test were not confirmed by GH peak after stimulation and/or IGF-I secretion. In such cases, the obtained data – in a part concerning the compatibility of the results of different diagnostic procedures – may not be fully representative of the whole population of short children.

Growth hormone concentrations were measured by hGH IMMULITE, DPC assay, calibrated to WHO IRP 80/505 standard, with the analytical sensitivity up to 0.01 ng/ml, the calibration range up to 40 ng/ml, the

Tab. 1. Number of patients with particular combinations of the results of screening test and stimulating tests.

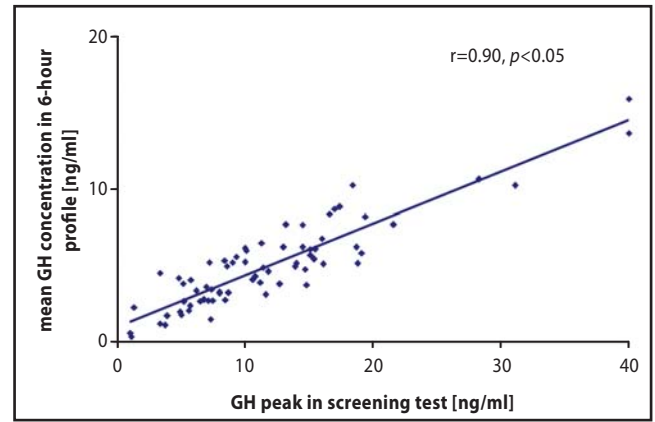
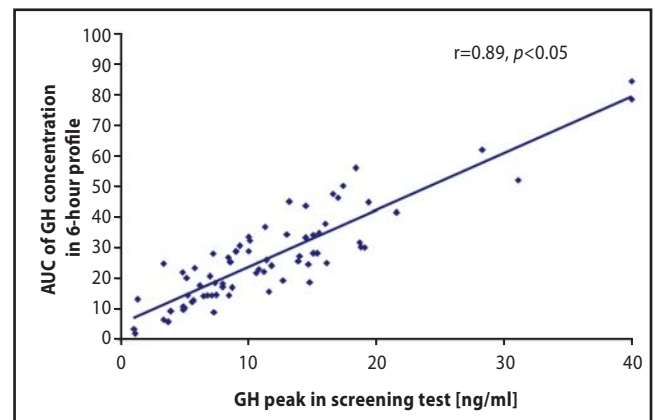
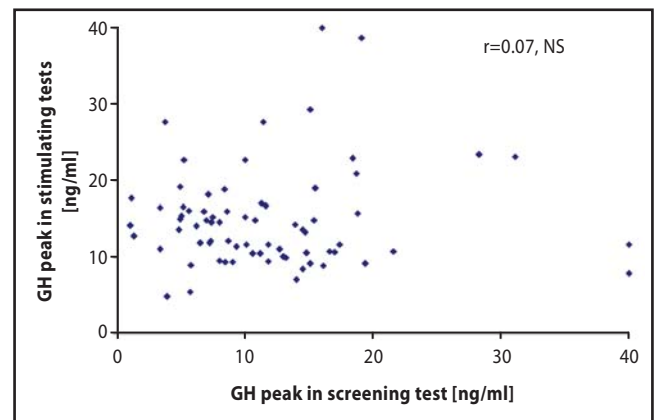
GH peak in screening test	GH peak in stimulating tests	
	subnormal <10 ng/ml	normal ≥10 ng/ml
subnormal <10 ng/ml	6 (8.3%)	27 (37.5%)
normal ≥10 ng/ml	7 (9.7%)	32 (44.5%)

sensitivity of 0.01 ng/ml, the intra-assay coefficient of variation (CV) – 5.3–6.5% and the inter-assay CV – 5.5–6.2%. The cut-off value for normal and decreased GH peak, both in nocturnal profile and in the stimulating tests, were assumed on the level of 10.0 ng/ml.

Serum IGF-I concentration was assessed by IMMULITE, DPC assay, with WHO NIBSC 1st IRP 87/518 standard, analytical sensitivity of the assay was 20 ng/ml, the calibration range up to 1 600 ng/ml, the intra-assay CV – 3.1–4.3% and the inter-assay CV – 5.8–8.4%. For comparison among the children with different age and sex, IGF-I concentrations were expressed as IGF-I SDS.

RESULTS

Very strong and significant correlation was found between GH peak during 6 hours after falling asleep and during first 2 hours of the same assessment, *i.e.* in the time period, fulfilling the conditions of screening test ($r=0.94$, $p<0.05$) (see Figure 1). Moreover, though extending the test duration from 2 to even 6 hours led to obtaining higher values of GH peak than during first 2 hours in 16 patients, in only 3 of them (4.2% of the studied group) normal GH peaks (>10 ng/ml) were observed – for the first time – later than during 2 hours of screening test. Thus, only in these 3 cases, the extending the test duration from 2 to 6 hours led to verifying the test result from positive (decreased GH peak) to negative (normal GH peak). The results of screening test correlated also with both mean GH concentration and the area under the curve (AUC) in 6-hour profile ($r=0.90$ and $r=0.89$, respectively, $p<0.05$) (see Figures 2 and 3, respectively). In the screening test, the mean value of GH peak was insignificantly lower than the maximal GH peak in 2 stimulating tests (11.7 ± 7.6 ng/ml vs. 14.9 ± 7.6 ng/ml), but, unfortunately, there was no correlation between GH peak in screening test and in stimulating tests ($r=0.07$, NS) (see Figure 4). The numbers of patients with normal or subnormal results of screening test and of stimulating tests are presented in Table 1. Thus, for the established cut-off level, the sensitivity of screening test was only 46.2%, while the specificity – only 19%. Certainly, these results cannot be applied for the whole population of

**Fig. 1.** The correlation between GH peak in screening test and the mean GH concentration during 6-hour nocturnal profile.**Fig. 2.** The correlation between GH peak in screening test and the AUC of GH concentration during 6-hour nocturnal profile.**Fig. 3.** The correlation between GH peak in the screening test and in stimulating tests.

short children, as only the patients with discrepancies between the results of different diagnostic procedures during previous assessments were subjected to the study. Moreover, no correlation was observed between IGF-I SDS and both GH peak in screening test ($r=0.17$,

NS) and any of the analyzed parameters of 6-hour nocturnal GH profile (for GH peak $r=0.17$, for AUC $r=0.14$, NS), as well as between IGF-I SDS and GH peak in stimulating tests ($r=0.09$, NS). Thus, GH peaks during 2-hour screening test seem to reflect overnight spontaneous GH secretion in children with short stature, remaining – however – discordant with both GH peaks in stimulating tests and IGF-I secretion.

Next, we attempted to assess whether it could be possible to shorten the screening test to less than 2 hours, preserving its credibility. For that reason the number of GH peaks exceeding the cut-off value for the first time during the screening test (that is considered as sufficient to confirm normal GH secretion after falling asleep) was assessed in particular time points. Normal results of 2-hour screening test were obtained in 39 out of 72 children (and in 3 other cases later – in 240, 270 and 300 minutes after falling asleep). Out of the analyzed 39 patients, in most (28 cases – 72%) normal GH peak was observed for the first time in 60 minute after falling asleep (1st sample), in 7 cases (18%) in 90 minute (2nd sample), in 2 cases in 120 minute and in 2 cases in 180 minute. Thus, only in 4 children (10%), the normal result of screening test was obtained later than in first 2 samples.

DISCUSSION

The relationships between spontaneous and stimulated GH secretion and the effectiveness of rhGH therapy in short children are still under discussion. Bercu *et al.* (1986) stated that the results of GH stimulating tests frequently did not reflect endogenous GH secretion. Moreover, in their study IGF-I levels correlated with mean 24-hour GH concentrations, suggesting that GH stimulating tests might not reflect endogenous GH secretion. However, in next few years, just the assessment of GH secretion in stimulating tests has become the most recommended procedure. In contrast, as mentioned before, Rosenfeld *et al.* (1995) questioned the legitimacy of the assessment of spontaneous GH secretion while diagnosing GHD. Similar was the statement of GH Research Society (2000). In consequence, the studies on spontaneous GH secretion in children with short stature during last 10 years have become scarce.

The starting point for analysis of our observations in the present study was the report of Rose and Munchichi (1999) who proved the accuracy of the assessment of GH secretion during 6 hours after falling asleep instead of 24-hour profile. On the other hand, we did not manage to find any data, directly justifying the adequacy of GH peak in 2-hour screening test as a surrogate of the assessment of spontaneous nocturnal GH secretion.

In different studies, either GH peak or the mean GH concentration, or the AUC of GH secretion during the selected time period were used as the indicators of spontaneous GH secretion. In some of them different

methods of GH pulsatility analysis were applied. However, these methods seemed to have limited utility for the purpose of our study, taking into account the small number of samples in the screening test. Thus, in our study, the selected parameters of 6-hour nocturnal GH profile have been compared with 2-hour fragment of the same test, fulfilling the principles of screening procedure. The very high correlations between the result of screening test and GH peak, and the mean GH level, as well as AUC of GH secretion in 6-hour test seem to confirm the adequacy of screening test for the spontaneous GH secretion assessment. Taking into account the fact that in 90% of patients, diagnosed as GH-sufficient on the basis of screening test, the first normal GH peak is observed in 60 or 90 minutes after falling asleep, it seems to be worthy to test the opportunity of shortening the screening test to the first 2 samples only. Moreover, Obara-Moszyńska *et al.* (2008), documented similar GH peaks in 30 and in 60 minute of sleep in a group of 56 prepubertal children with short stature. Further studies on that issue seem to be necessary to optimize the protocol of that procedure. The most important limitation of such studies is the necessity of observing the exact moment of falling asleep. Van Cauter *et al.* (1998) recommended that the test of nocturnal GH secretion should be referred to a sleep phase, as spontaneous GH peaks are observed mainly during the non-REM phase of sleep. However, such standards are impossible in practice to meet for the commonly used screening test. Taking into account the rule that screening procedures should be simple and relatively cost-effective, it seems that shortening the duration and optimizing the time points of the test of GH secretion after falling asleep may improve the diagnostic standards.

Another problem that should be a subject of further studies is the cut-off value for screening test. The studies on problem in question have not been carried out in other countries, as the assessment of GH secretion after falling asleep has not been recommended as a diagnostic tool. Thus, very interesting is the previously quoted study of Obara-Moszyńska *et al.* (2008). The authors stated that GH peaks after falling asleep were much higher than those obtained during stimulating tests, nevertheless regarding as appropriate the arbitrarily established cut-off value of GH peak in screening test on the same level as for the stimulating tests. In our study, the mean value of GH peak in screening test was slightly lower than in stimulating tests. However, it should be emphasized that the sensitivity of screening test (with respect to the diagnosis based on the results of stimulating tests) was only 46%. Preliminary results of our studies (Smyczyńska *et al.* 2008) suggested that the cut-off value for screening test, ensuring its high sensitivity should be higher, unfortunately, becoming associated with a very poor test specificity.

The lack of correlation between IGF-I concentrations and GH secretion (both spontaneous and stimulated) seems to be another problem, especially for GHD has

recently been classified as a form of secondary IGF-I deficiency (Wit *et al.* 2007) and the assessment of IGF-I secretion together with growth rate has recently been proposed as an improved screening for GHD (Lemaire *et al.* 2009).

The last but not least important issue seems to be the relationships between the results of different tests and the growth-promoting effect of rhGH therapy. Independently from the results of stimulating tests, better effectiveness of rhGH therapy in children with decreased spontaneous GH secretion was reported by Radetti *et al.* (2003). In the same year, Rogol *et al.* (2003) found that overnight serial sampling might be effective in predicting growth response only in case of severe GHD, being less useful in other patients.

Thus, it is possible that the arguments pointing at the advantage of pharmacological tests vs. the assessment of spontaneous GH secretion may be not as strong as it was previously regarded. Further observation of short children with assessed nocturnal GH secretion, both treated with rhGH and untreated, seems very interesting and important for optimizing the assessment of GH secretion and for verification, which tests (if any) are the best predictors of growth response to rhGH therapy. Currently, we are convinced that despite the fact that GH peak in 2-hour GH profile after falling asleep reflects spontaneous GH secretion, it does not fulfill the requirements for screening test, until GH stimulating tests remain a diagnostic standard.

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