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ANALYSIS OF FOUR-HOUR GROWTH HORMONE PROFILES AFTER ONSET OF SLEEP IN NORMAL AND GH-DEFICIENT CHILDREN: A STANDARDIZATION OF THIS SIMPLE TEST FOR CLINICAL USE

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SUMMARY

The study comprised three groups of 131 prepubertal children. Group A included 55 healthy children and Group B included 66 children with GHD. Group C included 10 children with growth retardation but normal GH response to pharmacological tests with flat GH-sleep testing compared with those of Group A.

All children except Group A underwent two pharmacological stimulation test for selection of patients. Spontaneous GH secretion was estimated by taking integrated 30 minutes blood samples for the first 4 - hour period of nocturnal sleep. The mean 4 - hour integrated GH concentration (ICGH), number of peaks (>5ng/ml) and the peak amplitude were calculated on 1048 occasions of sampling. The sensitivity of sleep test was defined by the percentage of true positive results.

The mean ICGH, peak number and peak amplitude in the control group were 5.77+1.22, 3.98 + 1.37 and 15.91 + 2.2 ng/ml respectively. The mean ICGH was 2.14+0.26 ng/ml in Group B and 3.12+0.6 ng/ml for Group C. There was concordance between GH response to pharmacological stimulation and short time sleep testing in 58 of 66 GHD patients. Thus the sensitivity of the test is 87.7 %.

In conclusion short time sleep test is safe, reliable and practical for clinical use. Four-hour sleep study should be especially indicated when GH responses to provocative testing are inconsistent with observed growth pattern. Long term sleep testing can be considered as a research tool rather than clinical one.

Key Words: *Neorosecretuary Dysfunction; Short Time Sleep Test.*

ÖZET

Derin Uykunun İlk 4 saatinde Büyüme Hormonu Profilinin Normal Çocuklarda ve Büyüme Hormonu Eksik olan Hastalarda Karşılaştırmalı Olarak Değerlendirilmesi: Uygulanımı Kolay ve Pratik Olan Bu Testin Klinik Kullanım İçin Standardizasyonu

Bu çalişmada normal çocuklarda derin uykunun ilk 4 saatinde büyüme hormonu (BH) profilinin değerlendirilmesi ve bu verilerin BH eksikliği olan hastalar ve nörosekretuvar disfonksiyonu olan hasta grubu ile karşılaştırılarak testin standardizasyonunu yapmak amaçlandı.

Çalışmada toplam 131 prepubertal olgu 3 grup halinde değerlendirildi. Grup A'da 55 sağlıklı çocuk, Grup B'de BH eksikliği tanısı alan 55 olgu ve Grup C'de boy kısalığı olan ancak farmakolojik uyarı testlerine yeterli yanıt veren 10 olgu değerlendirildi. Grup A dışındaki tüm olguların en az iki farmakolojik uyarı testiyle BH yanıtları değerlendirildi. Uyku profili sırasında 30 dakikalık aralarla 4 saat boyunca örnekler alındı. Ortalama 4 saatlik BH yoğunluğu (ICGH) pik sayısı (>5ng/ml) ve pik amplitüd toplanılan 1048 örnek üzerinden hesaplandı. Testin sensitivitesi Grup B'nin sonuçları ile karşılaştırılarak gerçek pozitif sonuçların yüzdesi ile hesaplandı.

Kontrol grubunu oluşturan Grup A'da sırası ile ICGH, pik sayısı ve pik amplitüdü 5.77(1,22, 3.98(1,37 ve 15.91(2,2ng/ml olarak bulundu. Grup B ve C'de elde edilen sonuçlar daha düşüktü. Ortalama ICGH Grup B'de 2,14(0,26 iken Grup C'de 3.12(0.6ng/ml idi.BH eksikliği olan 66 olgudan 58'nin uyku profili sonuçları farmakolojik uyarı testi sonuçlarıyla uyumluydu (sensitivite %87.7).

Kısa süreli üyku testi güvenilir, uygulanımı kolay pratik bir testtir. Büyüme yanıtları ile farmakolojik uyarı testi yanıtları uyumsuz olan olgularda 4 saatlik uyku profili özellikle denetlenmelidir. Uzun süreli uyku testleri kullanım zorlukları açısından günlük klinik uygulamadan çok araştırmalarda tercih edilmelidir.

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The accurate diagnosis of growth hormone deficiency (GHD) is essential before a patient is committed to long-term growth hormone (GH) therapy. Since the use of solely auxological criteria to GHD is unreliable, laboratory assessment of GH secretion induced by pharmacological and physiological stimuli has facilitated the diagnosis of GHD. Physiological impairment of GH secretion may well be more important than pharmacological deficiency (1,2). Investigations performed in the assessment of GH secretion in short children should be safe, reliable and easy to be carried out. Most of the investigators agree that the sampling for 12-24 hours is not practical and routine for evaluation of short stature (3,4,5,6). Night time profiles of GH secretion have been evaluated as investigative tools for the assessment of endogenous secretion (1-22). There is no any agreement for standardization of method for evaluation of GH sleep profiles (duration of sleep, with or without EEG monitoring, sampling intervals and cut off points) (1-24, 25, 26). In this study, GH secretory capacity of prepubertal healthy Turkish children with normal statural growth was investigated in the first 4 hours of nocturnal sleep in order to standardize this diagnostic procedure, and results were compared with those of GH-deficient children in the same age group.

SUBJECTS AND METHODS

Study Groups: The study comprised three groups of 131 prepubertal children.

Group A: A total of 55 healthy children (24 girls, 31 boys) with no statural retardation aged 8.60 ± 2.10 years. Their bone ages were 8.90 ± 1.00 years.

Group B (Idiopathic GH deficient group): A total of 66 (19 girls, 47 boys) growth retarded euthyroid children (height below -2.5 SD for chronological age) with subnormal height velocity (HV below 25th percentile for age); delayed bone age (above 2 years) and subnormal GH (below 7 ng/ml for complete GHD (n:58); 7-10 ng/ml for partial GHD (n:8)(response to two pharmacological tests. Organic brain disorders were excluded by the MRI and/or CT scan of the central nervous system.

Group C: Ten children (3 girls, 7 boys) with growth retardation just like those of Group B but normal GH (above 10 ng/ml) response to two pharmacological tests. Selection of this group was made after the results of sleep testing of group B were obtained (ICGH < 3.33ng/ml and/or peak amplitude < 11.5). Twenty seven patients with concordant response to pharmacological and sleep testing (idiopathic short stature or Kowarski syndrome) were excluded. Informed consent were obtained from all of the patient's parents for this study.

All children of the three groups were well nourished, euthyroid (with total and free thyroxine, basal TSH and TRH stimulated TSH levels) and had no hepatic, renal, cardiopulmonary, and gastrointestinal problems. None had skeletal dysplasia or dysmorphic syndromes. The height of each child was expressed as the SD score, i.e. height in relation to the sex and age matched Turkish standards (27). Bone age was estimated according to the method of Greulich and Pyle (28).

All the children except Group A (control group) underwent provocative tests for GH secretion with at least two pharmacological stimuli [insulin induced hypoglycemia (IIH 0,1 U/kg) and L-dopa (20 mg/kg)]. Complete GHD was diagnosed if the peak GH concentration was below 7ng/ml; partial deficiency if the peak concentration was 7-10 ng/ml and normal if the peak concentration above 10 ng/ml (24).

Spontaneous GH secretion was estimated by taking integrated 30-minutes blood samples for the first 4-hour of period nocturnal sleep without EEG monitoring. All of the subjects were evaluated for sleep-induced GH secretion on 1048 occasions of sampling. Children stayed at the hospital at least one night. They went to sleep at their own choxn times. Samples were drawn from an indwelling venous catheter, which was inserted prior to the onset of sleep. Nursing personnel

recorded each subject's status as awake or as sleep. Serum was separated within one hour of blood collection and stored at 40 C until being sent to laboratory the following morning. Plasma GH concentrations were measured by double antibody RIA, using DPC kits (1 ng/ml = 2mU/L). Intra- and interassay coefficients of variations were 5 and 7 %, respectively. The sensitivity of the GH assay varried between 0.5-1ng/ml.

Pulse Analyses: The mean 4-hour integrated GH concentration, number of peaks (GH > 5ng/ml) and the peak amplitude (highest nocturnal peak) were calculated for the first 4 hours of nocturnal sleep on 1048 occasions of sampling. Values of below 1 ng/ml were assigned as 1ng/ml (3).

Statistics: Results were expressed as mean + SD. Lower and upper normal limits are defined as + 2SD from mean. Compression of the mean concentration of GH, peak amplitude and peak frequency of groups were made using Student's "t" tests.

The sensitivity of sleep test was defined by the percentage of true positive result obtained when test was applied to patients known to have GHD confirmed by IIH and L-dopa testing in group B.

RESULTS

Evaluation of the first 4-hours GH profiles in the control group (Group A): The mean concentration of GH (ICGH), GH peak amplitude and peak number were 5.77 + 1.22 ng/ml, 15.91 + 2.20 ng/ml, and 3.89 + 1.37, respectively (Table 1). The lower normal limits of GH secretory profiles were 3.27 ng/ml for the GH concentration, 11.50 ng/ml for the peak amplitude, 1.15 for the peak number. The upper normal limits were 8.26 ng/ml for the GH concentration, 20.30 ng/ml for the peak amplitude, 6.63 for the peak number among 8 measurement. None in the Group A had the mean sleep GH concentration below 3 ng/ml . While only two subjects, (3.63 % of subjects) had the mean GH concentration above 8 ng/ml, most (96.37 % of subjects) had values between 3 and 8 ng/ml.

Evaluation of GH reserve in Group B: On baseline data the mean peak GH values obtained after L-dopa and IIH were 4.46 +1.50 ng/ml and 5.70 +0.85 ng/ml, respectively, in selection of GHD children. Pulsatile GH in this group was blunted and found to be significantly different from that of the control group as expected. The mean GH concentration was 2.14 +0.60 ng/ml, and significantly different from that of control group ($p<0.01$). Maximum value of integrated concentration of GH of Group B was below the lower limit of the control groups. This characteristic was also valid for other parameters of pulsatile GH secretion such as peak amplitude (6.16 +1.10 ng/ml) and peak number above 5 ng/ml (1.00 + 0.26). All of the 66 GH-deficient patients except 8 had concordant results from pharmacological tests and nocturnal sampling. So that short time sleeping test is diagnostic for GHD just as pharmacological testing in this group. The sensitivity of sleeping testing was 87.7 %.

Growth hormone reserve in Group C: The peak values of L-dopa and IIH tests were always >10 ng/ml, 14.02 +3.30 ng/ml for the first test and 12.72 +4.40 ng/ml for the second test, in selection of this group patients. The mean GH concentration, GH peak amplitude and peak

Table 1: The clinical characteristics and GH-reserve of groups.

Group A: Control

Group B: Growth retarded patients with blunted GH response to pharmacological stimuli

Group C: Growth retarded patients with normal GH response to pharmacological stimuli

ICGH * : Integrated concentration of GH

( )** : Lower normal limit - Upper normal limit

number were low: at 3.12 +0.63 ng/ml/4hr, 6.60 +1.23 ng/ml and 2.14 +1.06 respectively, similar to those of GH deficient children. This group of patients could be diagnosed as GH neurosecretory dysfunction (GH-NSD) by short time GH sleep testing.

DISCUSSION

In this study a practical method of sampling and analysis of 4 hour nocturnal GH profiles in prepubertal children was reevaluated for standardization of the method. Analysis of test performance was confined to the 55 healthy prepubertal children without growth retardation, in comparison with 76 prepubertal patients with short stature (66 GH deficiency, 10 growth retardation with normal GH response to pharmacological stimuli). Normal GH response to pharmacological stimuli may not always prove that spontaneous GH secretion is adequate (1,2,12). Children with GH-NSD may bear a clinical resemblance to subject with GHD but their response to provocative tests are normal. However, spontaneous GH secretory profiles in these children are abnormally low (2).

GH profiles have been widely studied in the last years and in this paper only few reports have been quoted among the references (1-23, 25, 29, 30,31). There is no any agreement for standardization of method for evaluation of pulsatile GH secretion. Some investigators have advocated the frequent sampling of GH during 24 or 12 hours period, but the clinical utility of this approach is controversial (2,3,20,25). The test with EEG recording of the deep sleep in children has been reported to be clinically useful (9,10,11,16,17). These studies showed that GH peaks occur with the onset and recurrence of slow wave stages of sleep (16). Previously clinical tests with sleep have concerned either single sampling after onset of sleep as screening procedure or greater sophistication than practicable in routine work such as EEG monitoring use of sleep laboratories (7,9,10,11,13,14,16,17). Frasier regarded sleep testing as a screening procedure in his review of GH testing in children, but he cited authors who had taken on one or two samples 60-90 minutes after sleep (31). King and Price sampled every 15 minutes from 30 to 120 minutes after clinical sleep onset, without EEG monitoring (14). They found low false-negative rate for the sleep test (5%) compared with arginine infusion (29%). Ward and Savage compared the peak serum GH concentration during first 5 hours of sleep with EEG monitoring with the serum GH response to insulin-induced hypoglycemia and arginine stimulation in 23 short children (16). Bierich described abnormalities in number of clinical condition associated with short stature by measuring serum GH concentration every 30 minutes during the first 5 1/2 hours of deep sleep (18). Most investigators agree that sampling for 12-24 hour is not practical and adequate for routine evaluation of short stature (6). As shown there is no any consensus about duration of short time sleep testing for evaluation of pulsatile GH secretion.

Also little information exist about the universal cutoff points of the mean GH concentration in physiological secretion for diagnosis of GHD and especially GH-NSD. Are the reported cutoff values for 24 hours or 12 hours of pulsation tests valid for short time sleep testing? Hypopituitary range of 24-hour integrated GH concentration was given as below 3.20 ng/ml (2,12).Costin et al assessed 24-hour GH secretion and GH response to provocative tests in 50 short statured children (none of them had complete GHD). They thought that those who failed to achieve a stimulated or sleep-induced GH peak of over 15 ng/ml and also had a 24 hour GH concentration less than 3 ng/ml may have some dysregulation in GH secretion (3). Lanes et al reported that normal values of GH during 9 hour sleep are 13 ng/ml for peak amplitude, 4.40 ng/ml for ICGH (5). Mori diagnosed GH-NSD in 6 short children with a mean GH concentration less than 5 ng/ml (22). Ward and Savage found that in those children who had normal GH reserve, the mean peak amplitude of serum GH during 5 hour sleep was 33.58 mU/ml (16.79 ng/ml). It has been reported that GH concentration during the first 3 hours of nocturnal sleep can be used instead of the 24 hour concen-

tration to assess spontaneous GH secretion (22,23). A cutoff point of 4 ng/ml for mean sleep GH was calculated from the correlation between mean GH values during 3 hours of nocturnal sleep and the mean value over 24 hours (The Japanese Criteria by the Foundation for Growth Science)(22,23). In this standardization, the children with idiopathic short stature were used as control group.

In our study control group for standardization of 4 hour sleep test include healthy, euthyroid, prepubertal Turkish children without growth retardation and bone age delay. The results of the control group were compared with those of GHD patients diagnosed by provocative testing.

Rose et al. questioned the diagnostic usefulness of spontaneous GH testing because they observed an overlap in mean 24 hr plasma concentrations of normal, short normal and GH-deficient children (20). In our study 66 GH-deficient patients (diagnosed with pharmacological tests) had very low GH levels compare to our results of the control group in sleep testing. In this group pharmacological stimulation tests and short time sleep test showed significant concordant results and the sensitivity was 87.7 %. There was a significant discordance between the results of pharmacological tests and nocturnal sampling in the group C. The diagnosis of growth hormone neurosecretory dysfunction could be obtained by short time sleep testing in this group.

In conclusion, short time sleep test is safe, reliable and practical for clinical use (sensitivity 87.7 %). Four- hour sleep study should be especially indicated when GH responses to provocative testing are inconsistent with observed growth pattern. On the other hand long term sleep testing can be considered as a research tool rather than clinical one.

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