Circadian pattern of prolactin secretion in children with growth hormone deficiency and congenital organic lesions in the hypothalamic-pituitary region

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Abstract

OBJECTIVES: Prolactin (Prl) secretion in children manifests circadian rhythm. The aim of the study was to assess circadian Prl pattern in children with growth hormone deficiency (GHD) and congenital organic disorders in the hypothalamic-pituitary region (HPR). **MATERIAL AND METHODS**: The analysis comprised 47 children (aged: 11.05±3.5 years) with GHD, divided (based on MRI) into subgroups: NORM (no disturbances in HPR); HP (pituitary hypoplasia) and PSIS (pituitary stalk interruption syndrome). The profile of circadian Prl secretion was determined, based on Prl measurements in serum every 3 hours during 24 hours. The macroscopic analysis of circadian Prl rhythm in particular groups was performed. The comparison group consists of 41 children (aged: 11.45±3.20 years) with idiopathic short stature (ISS). **RESULTS**: In GHD-HP, diurnal and nocturnal Prl concentrations were low but with the dispersion between them and with normal rhythm in most of cases. In GHD-PSIS, diurnal and nocturnal Prl concentrations were on the same level and the rhythm was not observed in most of cases. No significant differences were found in Prl secretions and Prl rhythm between GHD-NORM and ISS. The rhythm of Prl secretion was disturbed in: 72.7% of children with GHD-PSIS, 23.5% – with GHD-HP, 10.5% with GHD-NORM and 7.3% with ISS, only. **CONCLUSIONS**: Congenital organic lesions of HPR are associated with quantitative disorders and changes of the circadian pattern of Prl secretion. In children with GHD without organic lesions of HPR, the circadian rhythm of Prl secretion was not different from that with ISS.

ORIGINAL ARTICLE

INTRODUCTION

In normal conditions, prolactin (Prl) secretion in children (similarly as in adults) manifests circadian pattern with higher serum concentrations during the night and with lower ones during the day (Sassin *et al*., 1972, Parker *et al*., 1973, Waldstreicher *et al*., 1996). It is well known, that the rhythm of Prl secretion is disturbed in case of organic disorders of the hypothalamic-pituitary region (HPR), such us brain tumours or injuries. On the other hand, the circadian rhythm of Prl concentration in children with growth hormone deficiency (GHD), depending on congenital organic disturbances in HPR, has not, so far, been analysed.

The adenohypophysis consists mainly of somatotrophs (50–60% of pituitary cells) and lactotrophs (20–50% of pituitary cells). Development of the pituitary gland is controlled by several transcription factors of the POU-homeodomain class, such as Ptx1, Ptx2, Hesx1 (Rpx) and LIM-dependent proteins (P-LIM, Lhx3 and Lhx4) (Sheng *et al*., 1997; Thomas *et al*., 1995). After Rathke's cleft formation, PROP-1 (Prophet of Pit-1) turn off the expression of Hesx1 gene, turning on the expression of Pit-1 gene, what influences the differentiation of lactotrophs, somatotrophs and the early subpopulation of thyreotrophs (Bozzola *et al*., 1999, Horseman 2001). Thus, the mutations in these genes are responsible for disorders of pituitary gland development, i.e., either pituitary hypoplasia (HP) (mainly, due to the deficit of somatotrophs), as well as for disorders of pituitary cells differentiation (Pfaffle *et al*., 1999).

For example, mutations in Pit-1 and PROP-1 genes are associated with HP and deficiency in somatotrophs, lactotrophs and thyreotrophs) (Frish *et al*., 2000, Ward *et al*., 1998). Moreover, in case of mutation in PROP-1 gene, LH, FSH and cortisol deficiency is observed, too (Asteria *et al*., 2000). In the cases with heterozygous deletion in Hesx1 a broad spectrum of congenital pituitary defects is observed, ranging in their severity from isolated GHD (IGHD) to multiple hormonal deficiency (MPHD) (Tajima *et al*., 2003, Thomas *et al*., 2001) while homozygous deletion in Hesx1 lead to severe nervous system defects, the absence of optic vesicles and very small adenohypophysis (septo-optic dysplasia – SOD). It has also been proven that the development of lactotrophs is connected with kinase-Cdk4 and target mutation in this gene leads to a small size of adenohypophysis and 80% deficiency of lactotrophs and somatotrophs, while the development of other pituitary cells are not disturbed (Moons *et al*., 2002). It is well known that in turn, the mutation in GHRH receptor gene leads to HP due to deficit of somatotrophs but with the normal number other pituitary cells (i.e., lactotrophs) (Murray *et al*., 2000).

Concerning the neurohypophysis, its hypoplasia is probably connected with damage of phosphate tyrosine receptor PTPsigma, coded by Ptprs gene (Wallace *et al*., 1999). In turn, ectopia of the posterior pituitary (EPP) is connected with abnormal migration process during embryogenesis and neurohypophysis may be localised in different parts of the pituitary stalk, depending on the degree of disorders (Kandemir *et al*., 2000, Triulzi *et al*., 1994). In patients with EPP, the structural abnormalities of the brain midline are observed, i.e., HP, corpus callosum agenesis, optic nerve hypoplasia, SOD and Chiari I malformation (Nagel *et al*., 1997, Pinto *et al*., 1997).

In many cases, beside HP and EPP, the disorders of the pituitary stalk (thinned, truncated) are confirmed in MRI examination. The three elements have been named the pituitary stalk interruption syndrome (PSIS). Fairly probably, PSIS is genetically determined (Siegel *et al*., 1995), however, neither was any mutation observed in GH, GHRH and GHRH receptor genes nor in Pit-1 and PROP-1 genes. Usually, PSIS is connected with MPHD.

The goals of the study included: an evaluation of the circadian pattern of Prl secretion in children with GHD and different organic congenital lesions in HPR; a comparison of obtained results with those in children with GHD but without changes in HPR region (visible in MRI examination) and with idiopathic short stature (ISS); an assessment of the diagnostic value of Prl profile in the studied cases.

PATIENTS AND METHODS

The analysis comprised 47 children with GHD (35 boys and 12 girls; aged from 4.5 to 17.5 years, mean ± SD: 11.05±3.5 years) and 41 children with ISS (25 boys and 16 girls; aged from 5.2 to 16.3 years, mean ± SD: 11.45±3.2 years), which were the comparison group. Together we exam 88 short children (60 boys and 28 girls; aged from 4.5 to 17.5 years (mean ± SD: 11.53±4.2 years).

Following the obtained medical history and laboratory investigations, no chronic diseases, especially concerning the gastrointestinal tract or the urinary system, were found in any of the children. During the period of examinations, none of the children revealed any signs of infection.

The study was approved by the Regional Committee for Studies in Human Subjects. The experimental protocol was explained to patient's parents and an informed consent was obtained.

Auxological studies

In each child, the actual body weight and height were measured. Based on the obtained values, the height standard deviation score (H_{SDS}) and the body mass index standard deviation score (BMI_{SDS}) were calculated. These are the relative indices, expressing the body height and BMI of examined child by the number of standard deviations (SD) from the mean value for the age and sex in a Polish population (Palczewska and Niedzwiecka, 2001). The children were qualified into the study if H_{SDS} was below –2.0.

Diagnostics of short stature

In each individual, routine laboratory examinations were performed within the diagnostics of short stature dur-

ing hospitalisation at the Department of Endocrinology and Metabolic Diseases of Polish Mother's Memorial Hospital – Research Institute in Lodz, Poland.

In each child, serum concentration of thyrotropin (TSH), free tyroxine (FT4) and basal cortisol concentrations were assessed. In cases with hypothyroidism and/or adrenal cortex insufficiency, the assessment of GH secretion was performed after appropriate supplementation. In peripubertal children the gonadotropins stimulating test with gonadotropins released hormone (GnRH) were performed and FSH and LH concentrations before and after stimulation were evaluated. On the basis of karyotype, the Turner syndrome was excluded in girls.

GH serum concentrations were measured during two stimulations tests: at 0, 30, 60, 90, 120 minute, following clonidine administration per os (0.15 μg/m^2 body area) and at 0, 90, 120, 150, 180 minute, following intramuscular glucagone administration (30 μg/kg body mass).

Growth hormone concentrations were estimated, using the immunometric method (IMMULITE, DPC, the sensitivity: 0.01 ng/mL, the intra assay CV was 5.3–6.5%, the inter assay CV was 5.5–6.2%).

According to the maximal GH values (GHmax), obtained in these two stimulation tests, the patients were divided into the following groups:

Group ISS – idiopathic short stature (GHmax ≥10 ng/mL);
Group GHD – growth hormone deficiency (GHmax <10 ng/mL).

In children with deficiency of more pituitary hormones than growth hormone, MPHD was recognized.

MRI examination

MRI examination was performed in all the patients and the presence of organic abnormalities and the height of the pituitary gland (PtH) were evaluated.

The pituitary height was determined in the anteroposterior projection by measuring the greatest distance between the superior and inferior borders of the gland. Pituitary measurements were compared with the normal values published by Argyropoulou *et al*., in 1991, and expressed as the number of standard deviations from the mean value for the height age (HA) of child ($PtH_{SDSforHA}$).

Pituitary hypoplasia was diagnosed when the $PtH_{SDSforHA}$ value was below –2.0. If, in MRI examination, we additionally observed ectopia or lack of neurohypophysis and/or invisible or thinned pituitary stalk, the child was qualified as PSIS. Thus, the group with GHD was divided into the following subgroups:

- GHD-NORM – no disturbances in the hypothalamic-pituitary region;
- GHD-HP – pituitary hypoplasia;
- GHD-PSIS – the pituitary stalk interruption syndrome.

Children with acquired GHD (due to brain tumours and injuries) were excluded from the study.

Estimation of prolactin concentration

For Prl assays all the subjects were admitted to the hospital at least 24 hours before the study. In each child, the profile of Prl circadian secretion was determined on the basis of Prl concentrations in serum, measured every 3 hours during 24 hours. Blood samples were collected at 08:00, 11:00, 14:00, 17:00, 20:00, 23:00, 02:00, 05:00 and 08:00 h. All the blood samples were left to clot for 45 minutes; serum was removed after centrifugation, and stored at –20 °C until assay. Prolactin concentrations were measured by the electrochemiluminescence method (ELICA, Roche, Elecsys®Systems 2010, the sensitivity: 0.47 ng/mL, in the range up to 470 ng/mL, the inter assay CV was 1.8–3.4%). All the measurements were performed at the Laboratory of Immunochemical Research of Polish Mother's Memorial Hospital – Research Institute in Lodz, Poland.

Based on the measured Prl concentrations during 24 hours, the following circadian rhythm parameters were calculated (macroscopic analysis) (Cugini, 1993):

- the mesor (the overall mean level),
- the median,
- the area under curve (AUC),
- the peak level (the maximal Prl concentration),
- the trough level (the minimal Prl concentration),
- dispersion (differences between peak and trough levels),
- the amplitude (the peak level and the mesor ratio),
- the mean nocturnal concentration (Xn), (the mean Prl concentration from three nocturnal time points: 23:00, 2:00 and 5:00 h),
- the mean diurnal concentration (Xd), (the mean Prl concentration from three diurnal time points: 11:00, 14:00 and 17:00 h),
- the Xn/Xd ratio,
- the regression index (the directional index, i.e., the index of the slope of the regression straight line in relation to the axis of ordinates).

On the basis on the results obtained from our previous work, we recognised the presence of normal circadian Prl rhythm if, at least, one of the following three criteria are fulfilled: amplitude >1.8779; Xn/Xd ratio >1.685; the regression index <–0.4107 (Stawerska *et al*., 2007).

Statistical analysis

The data were statistically analysed, using the one-way analysis of variance (ANOVA), followed by post-hoc testing of the differences of means (RIR Tukey test). In certain cases, the non-parametric Kruscal-Wallis test was used for a screening evaluation of the differences of means. The frequency of disturbed Prl rhythm in particular groups was analyzed with a $\chi 2$ test and a Fisher's exact test. Statistical significance was determined at the level $p<0.05$.

RESULTS

The age of children and their BMI_{SDS} values were not different among the analysed groups but the children with GHD-PSIS and GHD-HP were statistically shorter than those with GHD-NORM. We found that the maxGH was statistically lower in GHD-PSIS than in GHD-NORM (certainly, in the ISS group GH concentration was normal and statistically higher in comparison with all other groups). We observed the lowest value of $PtH_{SDSforHA}$ in GHD-PSIS and GHD-HP groups, with statistical differences in comparison to the GHD-NORM group and the ISS group (Table 1).

Table 1. The mean values (±SD) of the chronological age (CA), growth deficiency (H_{SDS}), the body mass index (BMI_{SDS}), the maximal GH concentration (maxGH) and pituitary high ($PtH_{SDSforHA}$) in particular groups of children.

	Group GHD n=47 (100%)			Group ISS n=41 (100%)
	GHD-NORM (mean ± SD)	GHD-HP (mean ± SD)	GHD-PSIS (mean ± SD)	(mean ± SD)
No of children	19 (40.4%)	17 (36.2%)	11 (23.4%)	
CA (years)	12.09±3.11	10.87±3.53	9.52±3.65	11.45±3.20
H_{SDS}	−2.14±0.30 [a,b]	−2.80±0.72 [a]	−2.96±0.91 [b]	−2.21±0.65
BMI_{SDS}	−0.36±1.56	−0.45±1.07	−0.25±1.44	−0.89±1.18
maxGH [ng/mL]	6.82±1.58 [c,d]	5.14±2.50 [e]	2.34±2.95 [c,f]	17.0±6.21 [d,e,f]
$PtH_{SDSforHA}$	−0.24±1.21 [g,h]	−2.83±0.92 [g,i]	−3.45±1.33 [h,j]	−0.17±0.95 [i,j]

a–j: p<0.05

Table 2. Prolactin concentrations at each time point in particular groups of children.

	Group GHD			Group ISS
Prl (ng/mL)	GHD-NORM (mean ± SD)	GHD-HP (mean ± SD)	GHD-PSIS (mean ± SD)	(mean ± SD)
08:00	11.31±5.52	11.91±5.48	11.45±6.83	15.56±10.74
11:00	6.19±4.07	6.64±5.91	9.30±5.73	9.75±4.31
14:00	8.42±5.11	6.36±4.34	9.29±5.53	9.90±5.04
17:00	8.24±3.48	7.11±4.02	10.00±5.24	9.13±5.91
20:00	7.90±3.24	10.03±10.44	9.43±5.12	10.24±6.70
23:00	10.71±6.33	9.59±6.27	9.42±5.07	13.01±10.31
02:00	19.33±9.00	12.31±9.01 [a]	11.87±6.10 [b]	25.69±11.05 [a,b]
05:00	15.48±8.51	10.93±5.24 [c]	11.04±5.70	20.30±9.28 [c]
08:00	10.71±5.37	12.62±8.39	10.57±6.29	15.73±10.34

a–c: p<0.05

The mean values (±SD) of Prl concentrations at each time point of the circadian profile in particular groups of children are shown in Table 2. The mean chronograms for each group are presented in Figure 1. Circadian Prl rhythm parameters (macroscopic analysis) for particular groups are presented in Table 3.

In the group of children with GHD and HP, in almost all particular time points of profile, Prl concentration was lowest among the groups, with statistically differences at 2:00 and at 5:00 between that group and ISS group (Table 2). This was the reason for the lowest value of mesor, median, AUC, Xn and Xd in GHD-HP group with statistically differences for the mesor, the peak level, Xn value and dispersion value between that group and the ISS group and for the trough level, amplitude and Xn/Xd ratio between that group and GHD-PSIS. Despite on the low mean values of Prl concentrations during the day and during the night (however within the normal range), the clear dispersion between nigh and day was observed. It was the reason that the amplitude and the Xn/Xd index were normal and not different in comparison with the ISS group. Only the regression index was significantly lower than that in the ISS group, indicating the only slight differences in the, otherwise, consequent decrease of Prl concentration in time (Table 3).

Summing up, in children with GHD-HP, Prl secretion was reduced during the day and during the night, however, the rhythm of Prl secretion was observed in most of cases (13, out of 17; 76.5%) (Table 4). Among four children (23.5%), in which the disturbances of Prl circadian secretion were confirmed, MPHD was observed in two cases (case 1 and 2) and isolated GHD in another two (case 6 and 15). Moreover, in these two children with disturbed Prl rhythm and MPHD, additionally, hypoprolactinaemia (Prl concentrations below normal value during the day and night) was observed, while in other children from that group the Prl concentrations at particular time points were within normal range (Table 4).

Some different observations were made in children with GHD-PSIS.

The value of Prl concentration at 2:00 (as well as Xn value) was also significantly lower than in children of the ISS group. The peak level was the lowest but the trough level was the highest in comparison with other groups (we observed statistical differences between GHD-PSIS and ISS for peak level and between GHD-PSIS and both GHD-HP and GHD-NORM for trough level). Thus, we observed only slight differences between nocturnal and diurnal Prl levels in that group. In consequence of this fact, the values of dispersion, the amplitude, the Xn/Xd ratio and the regression index were significantly lower than in all the other analysed groups. We did not observe any rhythm of Prl secretion in most of cases from that group, although the mean values of Prl concentration at particular time points were within the normal range (Table 2).

Among the 11 children, qualified into that group, isolated GHD was observed in 6, while MPHD – in 5 children. In that group there were 5 patients with invis-

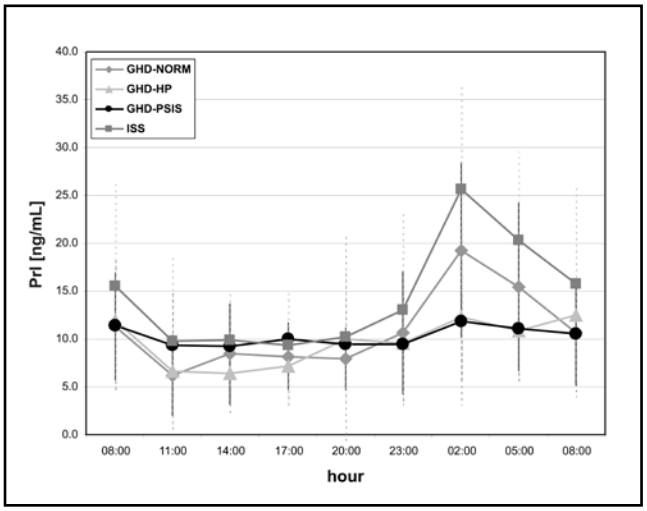

Figure 1. Chronograms of particular groups of the analyzed children.

ible pituitary stalk and 6 – with thin pituitary stalk in MRI scans. None of the invisible stalks became detectable after contrast administration. In that group, there were 7 patients with ectopia of neurohypophysis and 4 patients with lack of any neurohypophysis signal in MRI scans (Table 5). Only in one girl, diabetes insipidus was recognized (case 1). In another child, beside PSIS, hypoplasia of the left optic nerve was observed (case 5). The circadian Prl rhythm was not observed in most of the cases (8, out of 11) of that group. In two children (cases 1 and 2) Prl secretion was very low (below the reference values) and with lack of circadian rhythm, in one of them an invisible pituitary stalk was demonstrated and a thin pituitary stalk in the other. In turn, elevated Prl concentration was observed in another child (case 11), in which neither the neurohypophysis nor the pituitary stalk was identified in MRI scanning. However, in this case, basic Prl was higher only at 8:00, while the results of all the other Prl measurements were within the normal range.

Table 3. Estimated parameters of Prl rhythm in particular groups of children.

	Group GHD			Group ISS
	GHD-NORM (mean ± SD)	GHD-HP (mean ± SD)	GHD-PSIS (mean ± SD)	(mean ± SD)
mesor (ng/mL)	10.91±2.68	9.71±4.79 [a]	10.27±5.66	14.39±4.43 [a]
median (ng/mL)	9.03±2.40	8.82±4.51	9.80±5.45	11.62±5.03
AUC (ng/mL/24 hours)	308.9±77.0	261.19±132.7	274.59±143.93	367.05±114.84
peak level (ng/mL)	23.22±8.22	18.34±11.29 [b]	12.39±6.34 [c]	31.26±12.74 [b,c]
trough level (ng/mL)	4.41±1.57 [d]	4.51±2.83 [e]	8.56±5.20 [d,e]	5.75±2.48
dispersion (ng/mL)	18.84±7.84 [f]	13.78±10.40 [g,h]	3.83±1.95 [f,h,i]	25.51±12.79 [g,i]
amplitude	2.12± 0.46 [j]	1.81±0.45 [k]	1.23±0.10 [j,k,l]	2.16±0.51 [l]
X_n (ng/mL)	15.14±5.86	10.71±6.25 [m]	10.78±5.58 [n]	19.67±6.43 [m,n]
X_d (ng/mL)	7.63±3.42	6.72±3.96	9.53±5.46	9.65±4.65
X_n/X_d ratio	2.38±1.20 [o]	1.78±0.80 [p]	1.18±0.14 [o,p,r]	2.43±1.26 [r]
regression index	–0.40±0.41 [s]	–0.15±0.11 [t,w]	–0.06±0.05 [s,t,u]	–0.63±0.69 [u,w]

a–w: p<0.05

A very interesting observation was the lack of any statistical differences between Prl concentrations at particular time points and parameters of Prl rhythm between GHD-NORM and ISS groups. In both groups, mean Prl concentrations were normal at particular time points and a strong rhythm of Prl secretion was observed with higher values at night and lower values during the day. The normal values of Prl rhythm indices (amplitude, the Xn/Xd ratio and the regression index) showed a normal rhythm pattern of Prl secretion in almost all

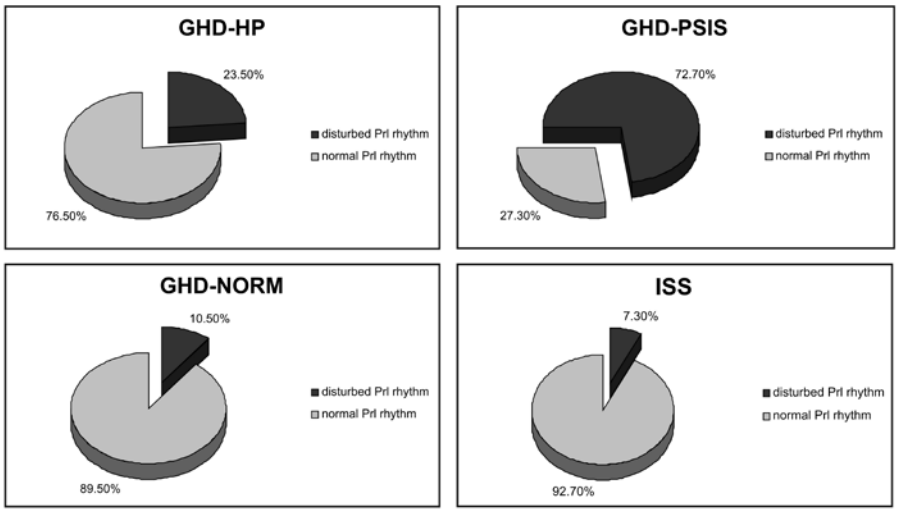

Figure 2. The percentage of normal and disturbed Prl rhythms in particular groups of children.

Table 4. Prl concentration at particular time points of circadian profile in patients from GHD-HP group.

	Diagnosis	Prl at particular time points [ng/mL]									Amplitude	X_n/X_d ratio	Regression index
		8:00	11:00	14:00	17:00	20:00	23:00	2:00	5:00	8:00			
1	MPHD	2.4	1.5	2.2	2.1	2.2	1.9	2.4	2.1	2.4	1.13	1.10	–0.01
2	MPHD	4.1	3.8	3.1	3.2	3.4	4.0	4.0	3.9	4.1	1.10	1.18	–0.02
3	IGHD	4.5	3.4	4.7	6.9	7.2	8.3	10.0	9.4	5.6	1.50	1.85*	–0.06
4	IGHD	5.9	4.2	4.1	4.5	3.8	6.6	12.3	10.0	5.9	1.93*	2.26*	–0.30
5	IGHD	6.1	2.8	2.2	2.9	3.0	8.1	7.3	5.2	4.5	1.73	2.61*	–0.05
6	IGHD	9.2	5.6	5.1	7.2	8.4	10.4	9.2	8.7	9.2	1.28	1.58	0.00
7	IGHD	11.7	2.5	3.0	4.9	4.6	4.5	4.3	12.0	10.0	1.88*	2.00*	–0.21
8	IGHD	12.1	5.1	5.5	4.0	9.6	18.7	23.7	14.6	10.8	2.05*	3.90*	–0.33
9	IGHD	12.6	7.2	5.3	9.2	8.9	9.9	17.5	13.0	12.6	1.64	1.86*	–0.34
10	IGHD	13.2	2.0	4.2	6.2	5.5	4.9	6.5	7.8	11.0	1.94*	1.55	–0.15
11	IGHD	14.8	2.6	6.1	18.6	24.7	25.8	28.2	20.2	20.4	1.57	2.72	0.05
12	IGHD	15.2	25.9	11.0	9.8	9.0	9.3	10.2	11.0	17.0	1.97*	0.65	–0.20
13	IGHD	15.9	4.9	3.9	4.3	5.0	6.6	6.6	7.0	15.9	2.04*	1.54	–0.18
14	IGHD	16.4	6.1	10.8	9.0	45.4	6.5	33.4	17.9	11.5	2.60*	2.23	–0.24
15	IGHD	16.9	14.9	18.6	13.1	15.3	19.0	21.4	19.5	15.4	1.25	1.29	–0.17
16	IGHD	20.4	11.3	5.9	6.3	10.9	15.6	8.4	15.6	39.1	2.64*	1.69*	–0.19
17	MPHD	21.1	8.7	13.3	9.2	2.5	3.2	3.3	7.0	18.0	2.20*	0.43	–0.20

IGHD – isolated growth hormone deficiency; MPHD – multiple pituitary hormone deficiency
Values marked * indicate the presence of Prl circadian rhythm according to established criteria (Stawerska *et al*., 2007).

Table 5. Prl concentration at particular time points of circadian profile in patients from GHD-PSIS group.

	NH	Stalk	Diagnosis	Prl at particular time points [ng/mL]									Amplitude	X_n/X_d ratio	Regression index
				8:00	11:00	14:00	17:00	20:00	23:00	2:00	5:00	8:00			
1	L	Th	MPHD+DI	2.6	1.8	2.5	2.7	2.4	2.7	3.7	3.2	2.6	1.38	1.37	–0.04
2	Ect	L	MPHD	3.6	2.7	2.1	2.9	2.6	2.4	3.4	3.3	3.6	1.22	1.18	–0.05
3	Ect	Th	IGHD	5.0	5.5	5.7	9.2	8.2	7.0	9.5	8.2	4.6	1.36	1.21	–0.02
4	Ect	L	IGHD	5.2	4.6	5.0	7.3	30.8	18.1	15.7	16.8	6.6	2.52*	3.00*	0.36
5	L	Th	MPHD+DPO	10.8	10.7	12.2	11.0	10.8	11.0	12.6	11.3	10.5	1.12	1.03	–0.04
6	Ect	L	MPHD	11.7	9.8	9.3	9.5	9.3	8.9	10.8	10.2	11.2	1.16	1.05	–0.10
7	Ect	L	IGHD	16.0	11.0	11.8	12.8	10.1	11.3	16.4	12.0	13.4	1.29	1.12	–0.20
8	L	Th	IGHD	20.8	15.3	13.1	15.1	14.8	15.2	17.8	19.3	17.0	1.26	1.20	–0.22
9	Ect	Th	MPHD	24.0	14.8	13.7	19.1	23.6	40.9	30.8	17.4	21.2	1.79	1.87*	0.36
10	Ect	Th	IGHD	24.0	21.5	21.3	20.7	20.4	19.7	24.2	22.1	24.0	1.10	1.04	–0.20
11	L	L	IGHD	30.8	13.0	20.1	11.1	12.2	21.3	31.4	33.3	23.4	1.52	1.95*	–0.86*

NH – neurohypophysis: E – ectopia; L – lack in MRI scans; Stalk: Th – thinned; L – lack in MRI scans; DPO – septo-optic dysplasia; DI – diabetes insipidus
Values marked * indicate the presence of Prl circadian rhythm according to established criteria (Stawerska *et al*., 2007).

children from these groups: 17, out of 19 from GHD-NORM group and 38, out of 41 from ISS group (Table 6 and Table 7).

Thus, an analysis of particular cases showed the lack of Prl circadian rhythm in 72.7% of children with GHD-PSIS, 23.5% of children with GHD-HP but only in 10.5% of children with GHD-NORM and 7.3% of children with ISS (the differences reveal statistical significances between GHD-HP and GHD-NORM, GHD-HP and ISS, as well as between GHD-PSIS and GHD-NORM and GHD-PSIS and ISS) (Figure 2).

DISCUSSION

In our group of children with GHD we observe the similar frequency of congenital disturbances (such as HP, PSIS and DPO) than in the literature; it can be stated that, regarding the children with GHD, about 50% include

Table 6. Prl concentration at particular time points of circadian profile in patients from GHD-NORM group

	Diagnosis	Prl at particular time points [ng/mL]									Amplitude	X_n/X_d ratio	Regression index
		8:00	11:00	14:00	17:00	20:00	23:00	2:00	5:00	8:00			
1	IGHD	4.2	5.2	19.5	5.2	10.5	7.6	22.9	24.7	8.2	2.06*	1.85*	–0.66*
2	IGHD	4.2	2.2	2.8	4.0	3.8	7.0	11.6	8.4	4.5	2.15*	3.00*	–0.22
3	IGHD	4.6	1.4	1.5	2.9	2.7	4.6	9.4	4.3	4.9	2.33*	3.16*	–0.19
4	IGHD	5.9	7.7	10.4	7.5	8.8	15.3	9.2	10.3	4.7	1.73	1.36	0.18
5	IGHD	6.8	3.8	8.8	7.2	5.9	21.9	33.8	30.1	5.7	2.45*	4.33*	–0.78*
6	IGHD	7.8	7.2	4.3	5.6	5.1	5.9	38.5	18.0	6.9	3.49*	3.65*	–1.19*
7	IGHD	7.9	4.2	6.1	6.5	16.0	3.8	11.9	11.1	18.4	1.93*	1.60*	–0.20
8	IGHD	8.3	6.2	8.2	9.4	7.9	6.6	37.5	20.2	22.0	2.67*	2.70*	–1.16*
9	IGHD	8.8	6.1	16.0	12.6	7.1	7.7	28.9	18.9	15.6	2.14*	1.60*	–0.78*
10	IGHD	9.7	5.3	8.0	10.6	3.6	11.0	16.4	8.0	6.0	1.88*	1.48*	–0.19
11	IGHD	9.8	8.1	6.0	7.0	12.0	22.9	17.5	6.0	5.0	2.19*	2.20*	0.26
12	IGHD	13.0	6.1	3.7	5.3	9.0	10.5	17.9	21.5	14.0	1.92*	3.30*	–0.56*
13	IGHD	14.8	3.5	5.1	6.6	8.5	22.8	18.8	17.0	11.4	1.89*	3.86*	–0.13
14	IGHD	15.3	3.3	5.0	5.0	5.5	12.1	16.2	36.8	17.8	2.83*	4.89*	–0.87*
15	IGHD	16.0	6.9	6.3	11.8	9.3	7.5	21.3	18.4	18.8	1.65	1.89*	–0.63*
16	IGHD	16.8	8.6	20.6	17.3	6.9	4.5	7.7	3.6	7.6	1.98*	0.34	0.08
17	IGHD	17.5	4.5	10.0	8.7	7.1	4.4	15.2	11.4	9.9	1.78	1.34	–0.42*
18	IGHD	20.0	5.3	7.0	10.7	11.2	17.9	15.6	13.3	9.9	1.63	2.04*	–0.02
19	IGHD	22.9	21.7	11.2	11.3	9.6	8.6	16.1	11.6	11.7	1.65	0.82	–0.36

IGHD – isolated growth hormone deficiency
Values marked * indicate the presence of Prl circadian rhythm according to established criteria (Stawerska *et al*., 2007).

cases of HP, while additional disorders of the pituitary stalk and of the nervous part of the pituitary gland are more rarely observed. Other disorders of the central nervous system structure, either as congenital malformations of the brain midline or Chiari type I malformations or DSO are rather sporadic among patients with GHD.

The way of pituitary size assessment in MRI imaging in children requires some explanation. As the pituitary volume changes with child age, we use the method, proposed by Argyropoulou *et al*., (1991). But following the results of our earlier studies (Hilczer *et al*., 2005), we have found that in case of children with short stature, it is more reliable to refer the obtained measurement results to the height age of child and not to the chronological age.

In our opinion, the most interesting aspect of this work is the evidence for differences in Prl circadian secretion models in children with GHD depending on the different congenital disorders (HP and PSIS) in HPR and revealing the strong relationship between the type of disturbances in HPR and Prl circadian rhythm abnormalities.

In some studies (Ward *et al*., 1998, Turton *et al*., 2005), concerning children with GHD-HP, only morning Prl concentration was evaluated but not circadian Prl concentrations. In most of cases the reduced basal Prl concentration were observed. In our group of children with GHD and HP, hypoprolactinaemia was observed in only 2, out of 17 cases (both in children with MPHD and without Prl circadian rhythm). In other children the Prl concentrations were low, but within normal range.

It was surprised, that in most of cases with HP (beside four children), the rhythm of Prl secretion exist and the values of amplitude and Xn/Xd ratio were within normal range. However the abnormal values of the regression index in all cases in that group showed that the pattern of Prl concentration were not entirely normal. In our opinion, it is connected with the predominant role of deficiency in lactotrophs and disorders in differentiation process but, perhaps, with some neurosecretion dysfunction, as well.

Among four children with HP and disturbed Prl rhythm, two of them demonstrated MPHD, while the other two presented with IGHD. This heterogeneity indicated that similar signs, i.e., HP and GHD contain inherent, different genetic disorders.

It seems, that if HP is connected with GHD only, and Prl concentrations are in normal range with present circadian rhythm, mutation in GHRH receptor gene may be suspected. If HP is connected with GHD and low concentration of Prl, mutations in Pit-1 or PROP-1 genes may be suspected. In turn, if HP is connected with

Table 7. Prl concentration at particular time points of circadian profile in patients from ISS group.

	Prl at particular time points [ng/mL]									Amplitude	X_n/X_d ratio	Regression index
	8:00	11:00	14:00	17:00	20:00	23:00	2:00	5:00	8:00			
1	3.8	4.3	3.9	2.9	4.4	14.1	16.0	10.0	4.5	2.25*	3.61*	–0.18
2	4.5	3.9	10.2	10.4	7.7	5.1	19.8	17.4	13.0	1.94*	1.73	–0.56*
3	5.4	4.4	4.1	2.7	8.6	2.7	19.5	24.4	17.8	2.45*	4.16*	–0.89*
4	6.3	4.7	5.4	4.6	7.4	4.0	30.9	15.8	14.5	2.97*	3.45*	–0.98*
5	6.4	5.1	7.3	9.5	9.0	9.7	23.0	17.2	12.7	2.07*	2.28*	–0.52*
6	6.6	6.1	11.6	9.2	11.9	7.5	17.5	14.4	26.9	2.17*	1.46	–0.40
7	6.7	5.9	5.7	7.7	6.0	6.5	15.3	12.6	7.5	1.86*	1.78	–0.37
8	7.0	5.0	19.9	8.6	4.7	7.9	17.9	13.2	6.3	1.98*	1.16	–0.36
9	7.4	7.8	16.0	12.4	10.5	6.5	44.6	29.3	16.0	2.67*	2.22*	–1.39*
10	7.6	11.2	17.6	20.5	15.3	14.8	28.8	23.7	20.8	1.61	1.36	–0.46*
11	7.7	4.8	10.1	9.9	8.7	5.3	41.2	38.7	10.0	2.72*	3.44*	–1.56*
12	7.9	11.6	8.8	6.3	5.5	12.8	15.6	9.8	10.5	1.58	1.43	–0.21
13	8.3	5.7	5.2	8.0	10.6	8.0	24.8	17.0	7.7	2.34*	2.63*	–0.60*
14	9.5	3.5	2.8	3.8	31.0	35.4	19.2	13.9	7.7	2.51*	6.76*	0.73
15	9.6	5.1	7.9	9.7	6.9	8.4	36.3	26.5	8.8	2.74*	3.14*	–1.15*
16	10.0	3.1	12.3	4.2	4.2	4.8	27.9	19.6	8.3	2.66*	2.67*	–0.97*
17	10.6	10.1	16.4	9.8	14.1	24.0	28.0	21.1	24.1	1.59	2.01*	–0.31
18	11.8	18.0	8.2	27.9	23.3	40.5	24.4	20.9	10.5	1.96*	1.59	0.66
19	11.8	18.0	8.2	27.9	23.3	40.5	24.4	20.9	10.5	1.96*	1.59	0.66
20	12.0	54.5	11.5	4.5	5.2	16.3	23.2	10.5	16.5	3.18*	0.71	–0.58*
21	12.5	6.0	7.1	6.2	6.0	11.7	15.5	14.6	6.1	1.63	2.17*	–0.31
22	13.2	15.9	11.6	8.7	7.7	15.6	15.6	10.1	7.0	1.36	1.14	–0.08
23	13.3	5.4	8.0	6.4	8.2	5.3	54.6	30.8	66.3	3.01*	4.56*	–2.20*
24	14.5	3.4	3.2	2.5	3.0	6.4	14.5	12.0	6.0	1.99*	3.62*	–0.50*
25	15.7	5.1	5.5	7.2	6.6	5.3	29.7	18.5	17.2	2.41*	3.01*	–1.02*
26	16.5	23.6	11.7	10.0	7.5	15.1	27.1	20.6	17.7	1.63	1.39	–0.72*
27	16.7	4.4	7.3	6.6	6.2	4.4	54.7	47.6	21.8	2.90*	5.83*	–2.36*
28	17.6	5.6	6.1	6.5	5.4	5.4	16.2	14.6	20.5	1.88*	1.99*	–0.63*
29	17.9	9.3	14.9	12.7	10.3	27.3	12.1	14.4	15.2	1.87*	1.46	0.32
30	18.6	10.0	5.6	11.0	11.9	32.6	24.4	12.8	10.0	2.14*	2.62*	0.15
31	19.9	4.4	5.1	6.0	5.5	20.7	19.7	12.3	11.7	1.77	3.41*	–0.22
32	22.9	10.9	27.0	6.1	17.9	19.0	12.3	7.5	9.3	1.88*	0.88	0.34
33	23.9	6.8	10.9	5.6	4.4	4.2	16.7	31.7	28.3	2.15*	2.26*	–1.12*
34	23.9	6.7	16.0	15.7	18.2	7.9	25.2	27.5	17.6	1.56	1.58	–0.69*
35	25.3	6.5	9.8	4.3	7.2	6.4	21.8	15.0	13.4	2.08*	2.10*	–0.75*
36	27.0	7.3	9.0	10.4	1.1	5.2	46.3	21.4	17.2	2.88*	2.73*	–1.68*
37	27.0	15.4	7.2	5.9	9.8	9.4	38.6	16.5	19.8	2.32*	2.26*	–1.18*
38	27.2	8.2	12.7	10.2	9.6	7.8	24.7	21.9	13.6	1.80	1.75	–0.82*
39	27.5	19.2	15.7	14.3	14.6	13.7	39.5	43.3	21.4	1.86*	1.96*	–1.42*
40	33.9	14.1	12.3	19.8	28.8	28.7	31.6	31.6	29.0	1.33	1.99*	–0.28
41	62.0	18.9	6.1	4.9	11.7	6.7	14.4	30.6	21.3	3.16*	1.73	–1.08*

Values marked * indicate the presence of Prl circadian rhythm according to established criteria (Stawerska et al. 2007).

deficiency of GH, TSH, LH, FSH, ACTH, but Prl concentrations are normal, with present circadian rhythm, then mutations in Hesx1 gene may be suspected. In the last case, severe nervous system defects, especially in midline may be observed, too.

Thus, observations of a circadian Prl secretion model are helpful in predicting the type of genetic disorder, which is responsible for HP and GHD.

Concerning lesions of the pituitary stalk, it should be emphasised, that after traumatic or postoperative transsection of the pituitary stalk, high Prl secretion is observed. It is connected with damage of dopaminergic neurons and blood vessels and the lack of appropriate inhibition of Prl secretion mainly by dopamine (DA). In case of PSIS, truncated stalk in MRI examination is also observed. However, it seems that a residual hypothalamic-hypopituitary connection is preserved even if it is not detectable with MRI (Chen *et al*., 1999, Liotta *et al*., 1999). Probably, the pituitary stalk is extremely thin with preserved vascular component but the neural component is lacking, indicating that the term "congenital agenesis of the neural pituitary stalk" is more appropriate than "pituitary stalk interruption" (Di Natale *et al*., 1994; Genovese *et al*., 1994, Maghnie *et al*., 1996). In consequence, in these cases the high Prl concentrations may be observed, just as in traumatic stalk interruption (Pinto *et al*., 1997).

We did not observe the increased Prl concentrations in patients from GHD-PSIS group (beside one with high Prl concentration at 8:00, only). Moreover, we observed reduced Prl concentrations during whole the day in two another cases from that group. We did not confirm any relationship between pituitary stalk visibility in MRI and the levels of Prl secretion in this group. So far, no similar studies have been performed. In Hanew *et al*., study (1991), the authors assessed only the basic Prl concentration and after TRH stimulating test. In those studies, the authors showed the lack of relationship between basic Prl concentration (or after TRH stimulation test) and the MRI image of pituitary stalk (normal, thinned, truncated). Moreover, the above-mentioned authors did not demonstrate any differences in basic Prl concentration (as well as after TRH stimulation) between the children with pituitary hypoplasia and those with normal size of the pituitary gland.

In our studies, no stimulation test with TRH for Prl secretion was performed, therefore, we could not compare the ours results with these obtained by Hanew *et al*., (1991), but it seems that the profile of circadian Prl secretion much better reflects the secretory function of lactotrophic cells in patients with PSIS than the test with TRH application.

Summing up, the disorders in circadian Prl secretion in GHD-HP are probably connected with insufficiency of lactotrophs with almost normal neurological regulation, while and in GHD-PSIS – mainly with abnormal neurological control. However, the normal anatomical connections between the hypothalamus and the pituitary gland with normal TIDA (tuberoinfundibular dopaminergic neurons), THDA (tuberohypophysial dopaminergic neurons), the median eminence, the stalk and the pars tuberalis are necessary for appropriate regulation of Prl secretion. In physiological conditions, the activity of TIDA neurons changes during the day, resulting in circadian differences of DA concentration in the median eminence and in the long portal pituitary vessels. The circadian rhythm of those neurons is of endogenic nature: higher DA concentration is observed during the day and lowers during sleep at night. Thus, the increase of Prl concentration during the day may at least partially – be resulting from diminished influence of DA on lactotrophs (Spiegel *et al*., 1994). It seems that in case of truncation of TIDA and THDA neurons and the lack of inhibiting influence of DA, Prl secretion should be higher all the day and night, such as after traumatic truncated of the pituitary stalk (Tyrrell *et al*., 1994). Thus in patients with PSIS and normal or low Prl concentrations, the mechanism of rhythm disturbances must be of another nature. It may be possible that damage of the stalk and defective functioning of dopaminergic neurons are overlapped by the predominant role of pituitary hypoplasia with deficit of lactotrophs and disorders of their secretion function. It may also be considered, that, in result of abnormal organogenesis, reduction of lactotrophs is observed only in part of the adenohypophysis. It is well known, that lactotrophs have functional heterogeneity, these from the external zone responding stronger to TRH stimulation than lactotrophs from the internal zone, while lactotrophs, sensitive to DA, are localised especially in the internal zone (Boockfor *et al*., 1987).

The studies have shown that the activity of TIDA neurons, beside their diurnal, light and darkness biased rhythm, is also characterized by a slow-paced rhythm, observed in constant darkness conditions. In turn, the activity of THDA neurons, although showing the diurnal rhythm pattern, changing in light and darkness, does not manifest any traces of the slow-paced diurnal rhythm. This difference, observed between the activities of TIDA and THDA neurons, when juxtaposed with the different pathways of DA transfer to the anterior pituitary lobe in either system, may, perhaps, be important for the low Prl secretion, as observed in PSIS, without maintained diurnal rhythm. These assumptions do require, however, further studies.

CONCLUSIONS

1. Congenital organic lesions of the pituitary gland are associated with quantitative disorders and changes of the circadian pattern of Prl secretion.
2. In children with GHD, without congenital organic lesions in hypothamic-pituitary region visible in MRI examination, the circadian pattern of Prl secretion was not different from the circadian secretion pattern of Prl in the children with ISS.

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