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Assessment of prolactin secretion in children: a profile of circadian prolactin secretion and the principles for interpreting it

Ocena wydzielania prolaktyny u dzieci: profil dobowego wydzielania prolaktyny i zasady jego interpretacji

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Abstract

Introduction: Prolactin (Prl) is secreted in a circadian pattern, although no method of interpreting it has yet been established. The aim of the study was to assess Prl secretion in children on the basis of the Prl circadian profile and to establish principles for the interpretation of the results obtained by this method.

Material and methods: The analysis comprised 41 healthy short children (25 boys); aged 5.2–16.3 years, in whom hormonal disorders and chronic diseases had been excluded. The children were divided into prepubertal or pubertal subgroups. Serum Prl concentrations were measured every 3 hours for 24 hours. To assess the rhythm the parameters of macroscopic analysis were calculated and receiver operating characteristic (ROC) analysis was performed. The group for comparison consisted of 30 children aged 8.9–17.2 years with hyperprolactinaemia.

Results: In each subgroup significantly higher Prl concentrations were observed at night than by day. No statistical differences were noticed between the groups regarding Prl concentrations at particular time points or parameter values during circadian Prl rhythm evaluation. In the group analysed weak correlations were found between age and Prl peak and trough levels. On the basis of ROC analysis criteria for the existence of normal circadian Prl rhythm in children were established.

Conclusions:

1. The presence of normal circadian Prl rhythm is observed if at least one of the following three criteria is fulfilled: amplitude >1.8779; X_n/X_d ratio >1.685; regression index <–0.4107.
2. No interpretation in relation to sex, age and stage of puberty is necessary for the circadian prolactin secretion rhythm in children.

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Key words: *prolactin, circadian rhythm, chronobiology, macroscopic analysis, children*

Streszczenie

Wstęp: Prolaktyna (Prl) jest wydzielana w rytmie dobowym, jednak dotychczas nie ustalono metody jego interpretacji. Celem pracy była ocena wydzielania Prl u dzieci na podstawie badania dobowego profilu Prl i ustalenie zasad interpretacji uzyskanych wyników badania.

Materiały i metody: Do badań zakwalifikowano 41 zdrowych niskich dzieci (25 chłopców); w wieku 5,2–16,3 lat, u których wykluczono zaburzenia hormonalne i choroby przewlekłe. Dzieci zostały podzielone na podgrupy ze względu na stadium dojrzewania płciowego. Stężenie Prl oznaczano w surowicy, co 3 godziny przez 24 godziny. W celu oceny istnienia prawi-

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dłowego rytmu Prl obliczono parametry analizy makroskopowej oraz przeprowadzono analizę *receiver operating characteristic* (ROC). Grupę porównawczą stanowiło 30 dzieci w wieku 8,9–17,2 lat z hiperprolaktynemią.
Wyniki: W każdej podgrupie stwierdzono znamiennie wyższe stężenie Prl w nocy niż w ciągu dnia. Pomiędzy podgrupami nie zaobserwowano żadnych znamiennych różnic w odniesieniu do stężenia Prl w tych samych punktach czasowych oraz wartości parametrów opisujących dobowy profil Prl. Ustalono istnienie słabej, znamiennej korelacji pomiędzy wiekiem kalendarzowym dzieci a maksymalnym i minimalnym stężeniem Prl w ciągu doby. Opierając się na analizie ROC, ustalono kryteria świadczące o istnieniu prawidłowego rytmu dobowego Prl u dzieci.
Wnioski:
1. Za istnieniem prawidłowego rytmu dobowego wydzielania Prl przemawia spełnienie przynajmniej jednego z 3 kryteriów: amplituda > 1,8779; współczynnik X_n/X_d > 1,685; współczynnik regresji < –0,4107.
2. U dzieci nie ma potrzeby stosowania innej, niż wymieniona, interpretacji wyników badania dobowego profilu Prl w zależności od wieku, płci czy stadium dojrzewania płciowego badanego dziecka.

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Słowa kluczowe: prolaktyna, rytm dobowy, chronobiologia, analiza makroskopowa, dzieci

Introduction

Assessment of prolactin (Prl) secretion is usually performed on the basis of fasting serum Prl concentration. In some cases a stimulation test after metoclopramide is performed, although there tend to be many false-positive results. When it is borne in mind that Prl secretion is modified by a number of external factors, such as light stimulus, stress, exercise, eating, hypoglycaemia, nipple irritation or uterine neck stimulation [1, 2], these results should be interpreted with some care [3, 4]. It seems that the most appropriate lactotrophic function is evaluation of physiological Prl secretion with circadian rhythm assessment. It has been demonstrated that in normal conditions Prl secretion manifests a circadian pattern, with lower serum concentrations during the day, which become about twice as high at night during the sleep [5–7]. However, no method of interpreting Prl secretion rhythm that could be useful for clinical purposes has yet been established. The aim of the study was to assess Prl secretion in children on the basis of the Prl circadian profile and to establish principles for interpreting the results obtained by this method.

Material and methods

The analysis comprised 41 healthy short children (25 boys and 16 girls), aged 5.2 to 16.3 years (mean ± SD: 11.45 ± 3.2 years), who had been diagnosed as being of short stature at the Department of Endocrinology and Metabolic Diseases in Łódź. Each child was prescribed the routine laboratory tests for the diagnosis of short stature. Hypothyroidism and adrenal cortex insufficiency were ruled out on the basis of normal values of serum thyrotropin, free tyroxine and cortisol concentration. On the basis of karyotype, Turner syndrome was excluded in girls. Growth hormone deficiency was excluded on the basis of normal values (above 10 ng/mL) of maximal growth hormone serum concentrations attained in at least one of two stimulation tests, one after clonidine administration *per os* (0.15 μg/m^2 body area) and the other after glucagone administration intramuscularly (30 μg/kg body mass). Growth hormone concentrations were measured in serum at 0, 30, 60, 90 and 120 minutes following clonidine administration and at 0, 90, 120, 150 and 180 minutes following glucagone administration. Concentrations of the insulin-like growth factor I were normal in each case.

After a medical history had been obtained and laboratory investigations carried out, chronic diseases, especially those concerning the gastrointestinal tract or the urinary system, were ruled out in all the children.

In each child current body weight and height were measured. On the basis of the values obtained the height standard deviation (H_{SDS}) and body mass index standard deviation (BMI_{SDS}) scores were calculated (the relative indices expressing the body height and BMI of each examined child by the number of standard deviations from the mean value for age and sex in the local population) [8]. In each child the stage of puberty was assessed according to Tanner's scale, which allowed the population of children to be divided into the following groups:
— Prepubertal girls (stage I);
— Prepubertal boys (stage I);
— Pubertal girls (stage II and III);
— Pubertal boys (stage II and III).

Moreover, each child's bone age (BA) was assessed on the basis of roentgenograms of the non-dominant wrist and hand according to Greulich and Pyle [9], and MRI examinations were performed. All children with any confirmed organic disorder of the hypothalamic-pituitary region (tumours, pituitary hypoplasia, pituitary stalk interruption syndrome or empty sella syndrome) were excluded from the study.

An analysis of Prl secretion was performed for each child on the basis of the circadian profile of serum Prl concentration, following comparison of Prl secretion in the subgroups, account being taken of sex and the stage of puberty.

Mode of evaluation of circadian Prl secretion profile

Assessment of Prl circadian profile was performed during the second day of hospitalisation, although a venous catheter was inserted into the forearm in the evening of the first day of hospitalisation to minimise stress-induced Prl release caused by the procedure itself.

On the day of the Prl assay no other diagnostic tests or any other examinations were scheduled. The children had their meals at the set times of 8.30, 10.00, 13.00, 16.00 and 18.30 and an isocaloric diet was recommended according to individual calorie requirements [10]. The patients were asked to abstain from exercise that day and not to take a hot bath during the hour before Prl measurement. The younger hospitalised children stayed with one of their parents in order to minimise stress associated with the hospital environment. The times when the light was switched off and when the children fell asleep and awoke were recorded in the study protocol. None of the children slept during the day. None of the children revealed any sign of infection at any time during the examination period. None of the children had taken any medication or received treatment which could have affected the Prl secretion pattern during the month before the examination

In each child the profile of Prl circadian secretion was determined on the basis of Prl concentration in serum measured every 3 hours over 24 hours. Blood samples were collected at 8.00, 11.00, 14.00, 17.00, 20.00, 23.00, 2.00, 5.00 and 8.00 h. All the blood samples were allowed to clot for 45 minutes; serum was removed after centrifugation, and stored at –20°C until assayed. Prl concentrations were measured by the electrochemiluminescence method (ELICA, Roche, Elecsys®Systems 2010; sensitivity 0.47 ng/mL in the range up to 470 ng/mL; inter assay CV 1.8–3.4%). All the measurements were performed at the Laboratory of Immunochemical Research, of the Research Institute, the Hospital of the Polish Mother, Łódź, Poland.

The study was approved by the Regional Committee for Studies with Human Subjects. The experimental protocol was explained to the patients' parents, who then gave their informed consent.

The methods applied for evaluation of the circadian Prl secretion profile

On the basis of the Prl concentrations measured over the 24 hours, the following circadian rhythm parameters were calculated (macroscopic analysis) [11]:

— the mesor (the overall mean level);
— the median;
— the area under the curve (AUC);
— the peak level (max);
— the trough level (min);
— the dispersion;
— the amplitude (the peak level and the mesor ratio);
— the mean nocturnal concentration (Xn);
— the mean diurnal concentration (Xd);
— 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);
— acrophase (peak time), the time point of the highest Prl concentration during the day;
— nadir (trough time), the time point of the lowest Prl concentration during the day.

Thus, in order to assess the existence of Prl circadian rhythm, three different indices were used:
— amplitude;
— X_n/X_d ratio;
— regression.

When the calculation methods of the area under the curve are taken into consideration, it should be stressed that the data obtained concerning the Prl concentration at particular time points of the diurnal profile were discrete values. Thus the waveform Prl secretion profile, according to the methodology described above, is a broken curve and not a polynomial continuous curve. This means that for this study the area under the curve may be determined on the basis of the classic formula of analytic geometry without the necessity of applying Fourier's analysis or, possibly, integrating the curves higher than the second order.

The possibility of applying cosinor analysis to the obtained data was also tested. It was shown that the values (at nine presupposed measurements) calculated from the theoretical harmonic curve ($f(x) = \cos(x)$) showed very weak compliance with the results obtained for the measurements. Concordance was tested by the least squares method and by tests of the compliance of the observed distribution with the theoretical. Thus it was decided to withdraw this method from the analysis.

Statistical analysis

In the statistical analysis for parametric variables, typical location and distribution measures (the mean, median, standard deviations and variance) were applied. Each of the parametric variables described in the Methods was submitted to a preliminary analysis of conformity with normal distribution with the use of the Komogorov-Smirnov and Lillefors tests. Regarding the normality of distribution of these variables, the analysis of variance (one-way ANOVA) was used in further parts

of the statistical analysis and the differences were tested by means of *post hoc* tests (because of different n values in particular trials Tukey's RIR test was used for the trials with unequal counts); in certain cases, the non-parametric Mann-Whitney U test was used for a screening evaluation of the differences of means. The analysis of parametric data correlation was performed on the basis of typical analysis of regression and correlation. A level of significance of $p < 0.05$ was accepted for all the tests and comparisons.

In order to compare the sensitivity and specificity of the indices evaluating Prl concentration variability during 24 hours the ROC curve analysis was employed, taking in the results of study of the group of children analysed and the results of studies on 30 children aged between 8.9 and 17.2 years (with a mean age of ± ± SD: 12.7 ± 3.1 years) with hyperprolactinaemia induced by pituitary adenoma or with hyperprolactinaemia accompanying the polycystic ovary syndrome and/or the metabolic syndrome.

Table II
Mean values (± SD) of Prl concentrations at particular time points in the circadian Prl profile in the group of children analysed

Tabela II
Średnie wartości (± SD) stężenia Prl w poszczególnych punktach czasowych profilu Prl w analizowanej grupie dzieci

Hour	Prl [ng/mL] mean ± SD	p < 0.05
8.00	15.56 ± 10.74	
11.00	9.75 ± 4.31	vs 02 and 05
14.00	9.90 ± 5.04	vs 02 and 05
17.00	9.13 ± 5.91	vs 02 and 05
20.00	10.24 ± 6.70	
23.00	13.01 ± 10.31	
2.00	25.69 ± 11.05	
5.00	20.30 ± 9.28	
8.00	15.73 ± 10.34	

Results

The group of children analysed consisted of 25 boys (17 boys at the prepubertal and 8 at pubertal stage) and 16 girls (all of whom were at the prepubertal stage). Table I presents selected anthropometric data for the group analysed and for particular subgroups.

In the group of children analysed, significantly higher Prl concentrations were observed at 2.00 and at 5.00 than at 11.00, 14.00 and 17.00 (Table II, Fig. 1). Circadian Prl rhythm parameters (macroscopic analysis) are presented in Table III.

On the basis of the ROC curve analysis the following values were calculated for sensitivity, specificity, the area under the curve and cut-off points for normal circadian Prl rhythm in healthy children:

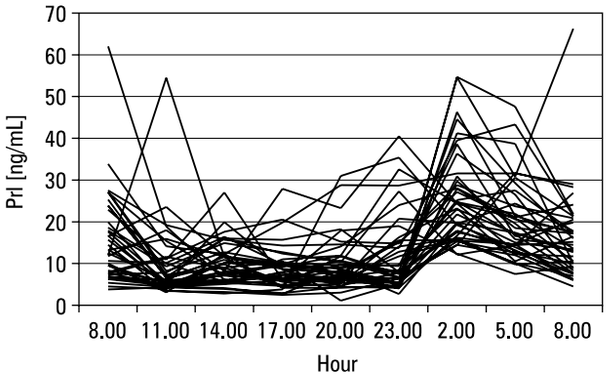

Figure 1. *Prl chronograms of all the children in the group analysed*
Rycina 1. *Chronogramy Prl wszystkich dzieci analizowanej grupy*

Table I
Mean values (± SD) of chronological age (CA), bone age (BA), height (H SDS) and weight (BMI SDS) in the group of children analysed and in individual subgroups

Tabela I
Średnie wartości (± SD) wieku chronologicznego (CA), wieku kostnego (BA), wzrostu (H SDS) oraz masy ciała (BMI SDS) w analizowanej grupie dzieci i w poszczególnych podgrupach

	Prepubertal girls (mean ± SD)	Prepubertal boys (mean ± SD)	Pubertal boys (mean ± SD)	Total (mean ± SD)
n	16	16	9	41
CA	9.91 ± 2.96	11.06 ± 2.76	14.91 ± 0.98	11.45 ± 3.20
BA	7.20 ± 0.85	8.42 ± 0.63	12.73 ± 1.16	9.33 ± 0.93
H SDS	–2.28 ± 0.78	–2.06 ± 0.36	–2.37 ± 0.80	–2.21 ± 0.65
BMI SDS	–0.98 ± 0.93	–0.92 ± 1.17	–0.66 ± 1.63	–0.89 ± 1.18

Table III
Mean values (± SD) of macroscopic analysis parameters calculated for the assessment of the circadian Prl profile in the group of children analysed

Tabela III
Średnie wartości (± SD) parametrów analizy makroskopowej wyliczanych w celu oszacowania dobowego profilu Prl w analizowanej grupie dzieci

	(mean ± SD)
Mesor [ng/mL]	14.39 ± 4.43
Median [ng/mL]	11.62 ± 5.03
AUC [ng/mL/24 h]	367.05 ± 114.84
Peak level [ng/mL]	31.26 ± 12.74
Trough level [ng/mL]	5.75 ± 2.48
Disperse [ng/mL]	25.51 ± 12.79
Amplitude	2.16 ± 0.51
X_d [ng/mL]	9.65 ± 4.65
X_n [ng/mL]	19.67 ± 6.43
X_n/X_d ratio	2.43 ± 1.26
Regression index	–0.54 ± 0.38

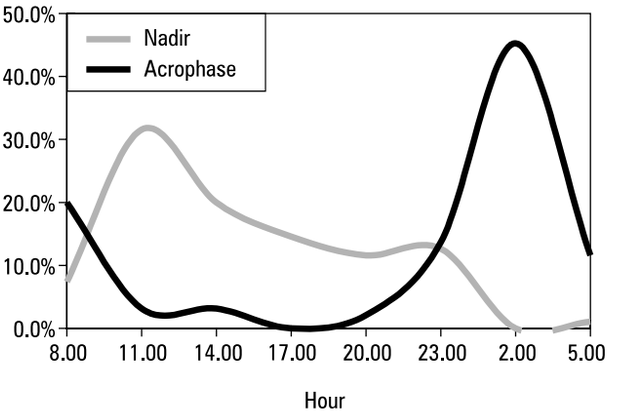

Figure 2. *The incidence of Prl nadir and acrophase at particular time points in the circadian Prl profile for the group of children analysed*

Rycina 2. *Częstość występowania nadiru i akrofazy w poszczególnych punktach czasowych dobowego profilu Prl w analizowanej grupie dzieci*

— for the amplitude: sensitivity = 80.0%; specificity = = 78.1%; the area under ROC curve = 0.861; criterion < 1.8779;
— for the X_n/X_d ratio: sensitivity = 93.3%; specificity = = 87.5%; the area under ROC curve = 0.922; criterion < 1.685;
— for the regression index: sensitivity = 90.0%; specificity = 67.2%; the area under ROC curve = 0.836; criterion < –0.4107.

The highest sensitivity (93.3%) index was that of the X_n/X_d ratio, although there were no statistical differences between any of the indices (p = 0.115).

A normal circadian Prl profile was observed in all the children analysed, indicating that at least one of the three criteria were fulfilled.

In each case, the peak time (acrophase) and trough time (nadir) of Prl concentration were established. It was found that in the group of children analysed the nadir was most frequently observed at 11.00, 14.00 or 17.00 (68.3% of cases), although, minimal concentration was also noticed at 20.00 (12.2%) and 23.00 (14.6%) but never during sleep (at 2.00 or 5.00). The acrophase was mainly observed at night: 23.00, 2.00 or 5.00 (73.2% of cases), although in some cases it was also noticed at 8.00 (17.1%), at 11.00 (4.9%) and at 14.00 (4.9%) but never at 17.00 and 20.00 (Fig. 2). On the basis of analysis of nadir and acrophase incidence rates at particular time points the conclusion was drawn that the comparison of nocturnal and diurnal Prl concentrations is the best method of analysis of the circadian Prl rhythm. However, it is also very important to assess circadian rhythm on the basis of other indices (amplitude and the regression index) also because in some cases the maximal and minimal Prl concentrations are observed at atypical time points, where the rhythm is normal but only somewhat shifted in time.

Analysis of circadian Prl profile in relation to age, sex and the stage of puberty

Analysis was made of differences between Prl concentrations at particular time points in the subgroups in relation to the patient's sex and the stage of puberty. The data are presented in Table IV.

The mean values of Prl concentrations at particular time points did not differ between particular subgroups. However, in each subgroup significantly higher Prl concentrations were observed at nocturnal time points than at the time points during the day.

The highest mean values of the amplitude, Xn/Xd ratio and the regression index were observed in the male pubertal group, although without statistical differences. No statistical differences were noticed either between groups regarding other parameters during circadian Prl rhythm evaluation (Table V).

In the group of children analysed weak but significant correlations were found between the chronological age (CA) and the peak Prl level (r = 0.24), CA and trough Prl level (r = –0.22), CA and Xn (r = 0.28) and between BA and peak Prl level (r = 0.27), BA and trough Prl level (r = –0.24) and BA and Xn (r = 0.31). It should be stressed that for the same parameters slightly stronger correlations were observed for BA than for CA.

Table IV
Comparison of the mean values (± SD) of Prl concentrations at particular time points of the circadian Prl profile in the subgroups identified

Tabela IV
Porównanie średnich wartości (± SD) stężenia Prl w poszczególnych punktach czasowych profilu dobowego Prl w wyodrębnionych podgrupach

Hour	Prepubertal girls Prl [ng/mL] (mean ± SD)	Prepubertal boys Prl [ng/mL] (mean ± SD)	Pubertal boys Prl [ng/mL] (mean ± SD)
8.00	14.36 ± 11.39	12.78 ± 8.10	15.46 ± 6.67
11.00	8.55 ± 6.18	8.89 ± 8.67	6.60 ± 4.87
14.00	9.75 ± 6.48	9.59 ± 4.97	6.60 ± 2.91
17.00	9.79 ± 6.86	8.94 ± 4.94	7.01 ± 2.64
20.00	11.64 ± 8.90	9.64 ± 6.41	8.92 ± 5.96
23.00	13.06 ± 11.08	12.61 ± 10.89	11.70 ± 8.07
2.00	22.49 ± 11.20	19.60 ± 10.93	21.04 ± 12.61
5.00	17.73 ± 9.13	16.00 ± 8.53	18.40 ± 11.06
8.00	14.92 ± 11.83	12.69 ± 7.01	13.55 ± 8.13

Table V
Comparison of the mean values (± SD) of macroscopic analysis parameters calculated for the assessment of the circadian Prl profile in the subgroups identified

Tabela V
Porównanie średnich wartosci (± SD) parametrów analizy makroskopowej wyliczanych w celu oszacowania dobowego profilu Prl w wyodrębnionych podgrupach dzieci

	Prepubertal girls (mean ± SD)	Prepubertal boys (mean ± SD)	Pubertal boys (mean ± SD)
Mesor [ng/mL]	13.59 ± 5.85	12.31 ± 5.34	12.14 ± 3.60
Median [ng/mL]	11.31 ± 5.75	10.66 ± 5.28	9.39 ± 2.88
AUC [ng/mL/d]	344.94 ± 143.21	334.30 ± 146.81	333.96 ± 107.05
Peak level [ng/mL]	28.05 ± 14.64	24.33 ± 12.53	26.48 ± 12.04
Trough level [ng/mL]	5.93 ± 3.68	5.93 ± 3.52	4.72 ± 1.74
Disperse [ng/mL]	22.12 ± 14.23	18.40 ± 11.55	21.77 ± 11.87
Amplitude	2.05 ± 0.58	1.95 ± 0.54	2.11 ± 0.43
X_d [ng/mL]	9.36 ± 5.28	9.14 ± 4.77	6.74 ± 2.92
X_n ng/mL]	17.76 ± 8.04	16.07 ± 8.16	17.05 ± 7.14
X_n/X_d ratio	2.18 ± 1.08	2.00 ± 0.98	2.88 ± 1.54
Regression index	–0.44 ± 0.59	–0.36 ± 0.48	–0.54 ± 0.69

Discussion

Assessment of Prl secretion on the basis of fasting morning Prl concentration or in a stimulation test (after metoclopramidum administration) may bring false results, especially in children, owing to the possibility of increased Prl concentrations induced by the stress on the child of the hospital environment in general or particular events such as blood sample collection. Thus the results should be interpreted with some care, especially if they are to be used in a screening test [3, 4].

It is well known that Prl is secreted in a circadian manner with higher secretion rates at night and lower ones during the day [5–7]. For the purpose of experimental studies the assessment of circadian Prl rhythm is performed by pulse analysis, which is connected with measurement of Prl concentrations in blood samples taken every 10–25 minutes throughout the day [12–14]. The results are submitted to computer analysis based on analysis of spectral resolution (Fourier analysis). Fourier analysis (cosinor analysis) is one of the most important methods of numerical signal processing (micro-

scopic analysis). It is used for "smoothing" the curves which oscillate around the main route. Thus the method is used for evaluation of numerous measurements performed in short time intervals [11, 15–18]. The other method of circadian rhythm analysis is non-inferential chronobiometry (macroscopic analysis). The macroscopic analysis is principally based on measuring the central location and dispersion of data obtained at each time point. The true effect of time on the temporal distribution of data may be ascertained by means of one-way variance analysis (one-way ANOVA) and the homogeneity of variance at each time point may be verified by means of statistical tests [11]. Whereas a relatively high number of blood sample collections are necessary for the microscopic analysis, the macroscopic analysis requires only a few blood collections and these are performed during a definite period of time. Thus it seems that for clinical purposes it is possible to use macroscopic analysis based on a few periodic measurements of Prl over 24 hours. In our work this method of circadian Prl rhythm analysis was used on the basis of nine Prl serum measurements over 24-hours (8.00, 11.00, 14.00, 17.00, 20.00, 23.00, 2.00, 5.00 and 8.00). The aim of the study was not the evaluation of the pulsatile manner of Prl secretion but to obtain information about the differences between Prl secretion rates observed during the day and at night. Thus for circadian Prl rhythm assessment it is very important to compare Prl secretion rates at these two periods and to establish whether the values of Prl concentration during the day are within the range and whether they increase at night in an appropriate way (approximately doubling) [5]. The acceptance of this assumption enables the diagnostic role of diurnal Prl secretion to be evaluated and to be regarded as a study which can be performed at any hospital without special equipment or software support. A weak point of the study is the fact that it provides only approximate evaluation of diurnal secretion and may thus lead to false results.

Other authors have also used a few Prl measurements to assess Prl circadian secretion in individuals under examination, taking into consideration the following patterns: every hour during a 24-hour period [19], four measurements during 24 hours, performed at 18.00, 22.00, 2.00 and 8.00 [20], every 4 hours from 20.00 to 8.00 [21], every 4 hours during the day and every 2 hours during the night (14.00, 18.00, 22.00, 2.00, 4.00, 6.00 and 10.00) [22], every 20 minutes over 4 hours during the day (10.00–14.00) and every 20 minutes over 4 hours at night (22.00–2.00) [23]. It should be stressed that most of the studies concerned a small number of children and macroscopic analysis was not performed.

In our study, we decided to determine the profile of Prl concentrations on the basis of nine Prl measurements in blood samples collected every 3 hours over a 24-hour period. In this way we obtained the following: three diurnal Prl measurements, in which Prl concentration should reach the trough level (11.00, 14.00 and 17.00); one evening measurement, when we could have expected a slight increase in Prl concentration (20.00); three nocturnal measurements (23.00, 2.00 and 5.00), in which Prl concentration should reach the peak level; two morning fasting measurements at 8.00 h, which are usually used for screening of Prl secretion. The advantage of this blood sample collection mode for Prl concentration measurements is its periodic character (every 3 hours) and the possibility of obtaining mean nocturnal and diurnal concentrations based on three subsequent blood samples. Taking into account the fact that the nadir is usually observed during the day and the acrophase at night, we calculated the mean diurnal concentration (11.00, 14.00 and 17.00) as the lowest level and the mean nocturnal Prl concentration (23.00, 2.00 and 05.00) as the highest level and compared them (X_n/X_d ratio). If Prl peak level was observed at a time other than at night, but the remaining measurements were within normal range, the value of amplitude increases, indicating the existence of rhythm. If the rhythm is entirely shifted in time, the existence of rhythm is confirmed by the regression index.

In the subgroups analysed (prepubertal girls, prepubertal boys and pubertal boys), the Prl circadian rhythm was observed with significantly higher (approximately doubled) Prl concentrations at night than during the day. This is in conformity with the data from the literature, as it is known that the circadian rhythm of Prl secretion forms in the first year of life and then reaches the values characteristic of adults, undergoing only slight modifications during puberty [20, 22].

Some investigators have indicated a relationship between Prl concentration, sex and the stage of puberty attained, but the results have been controversial [12, 22, 24–29]. Beck and Wuttke [20] did not observe any statistical differences in fasting Prl concentrations in 82 healthy children at particular stages of puberty, while the values were comparable with Prl levels observed in adults.

In turn, Dahlgren et al. [22] observed no statistical differences in Prl secretion at 14.00, 18.00, 22.00, 2.00, 4.00, 6.00 and 10.00 in prepubertal children in relation to sex, age, height and weight, although the authors noticed that in healthy pubertal boys Prl concentration is lower than in prepubertal boys. On the other hand, it is higher in healthy pubertal girls than in pubertal boys. In the group of children analysed no statistical differences were observed in Prl concentrations between prepubertal and pubertal children at particular time points, but some discrete differences were noticed.

It should be underlined that in our group of patients no pubertal girls were involved, and there were more children without signs of puberty than in puberty, which may have influenced our results.

We observed, in turn, a positive correlation between CA and Prl concentration at 2:00 and at Prl peak level. The same correlation was observed for BA. In the group of children analysed the values of the amplitude increased gradually with age for boys, while in girls the highest values were observed at the age of 8–10 years, decreasing after that time. This was in agreement with other reports from the literature [20]. The rise of Prl secretion in girls at the late prepuberty stage may have been associated with the beginning of pulsate luteinising hormone secretion. Similarly, the rise in Prl secretion during puberty is connected with the rise in luteinising hormone secretion [30].

Gassler et al. [31] presented the range of normal morning concentrations of Prl, luteinising hormone, follicle-stimulating hormone, testosterone and oestradiol as measured in 299 healthy children divided into eight different age groups. The authors observed slightly higher Prl concentrations in girls than in boys in each age group. This may have been connected with differences in the circadian signal from the central nervous system in both sexes associated with different hormonal (i.e. oestradiol) levels during the prepubertal and pubertal periods in boys and in girls.

It should be emphasised that the above-mentioned clinical studies of Prl secretion in children and adults usually did not present any criteria for the interpretation of the circadian Prl profile. In our work we demonstrated a useful tool for evaluation of Prl secretion in children. Our criteria make it possible to evaluate circadian Prl rhythm, not only when Prl concentration is highest at night and lowest at day, but also in these cases in which acrophase or nadir are shift. This may be linked to sleep difficulties or stress, but a circadian rhythm exists and this is normal.

To sum up, our results indicate that the sex of the child and the stage of puberty reached did not significantly change the circadian Prl rhythm in healthy short children. It was not therefore necessary to create different ranges of normal values for the parameters used to evaluate the circadian Prl rhythm for boys and girls either for prepubertal or for pubertal children.

The amplitude of Prl concentrations increased at night, together with CA (and BA), although the correlations calculated were not strong, indicating that the age of child had only a weak influence on nocturnal Prl secretion. Thus neither CA nor BA has any significant influence on Prl secretion and no separate interpretation is necessary of the parameters evaluating the circadian Prl rhythm in particular age groups.

On the basis of the ROC analysis it was demonstrated that the indices applied for the assessment of the circadian Prl rhythm (amplitude, Xn/Xd ratio and the regression) are characteristic of high sensitivity and specificity with no statistical differences between them. The normal circadian Prl rhythm was present if at least one of the following three criteria was fulfilled: amplitude > 1.8779, X_n/X_d ratio > 1.685 and regression index < -0.4107.

The analysis of the incidence of minimal and maximal Prl concentrations as observed at particular time points demonstrates that although the best method of circadian Prl rhythm assessment is based on the Xn/Xd ratio, the Prl rhythm should also be evaluated according to the other two criteria (the amplitude and the regression index), because in same cases the peak and trough Prl levels are present at atypical time points with the Prl rhythm is present and showing only some variations of time.

Conclusions

1. In healthy children, Prl secretion demonstrates a diurnal pattern, with higher concentrations at night than during the day.
2. The presence of normal circadian Prl rhythm is observed if at least one of the following three criteria is fulfilled: amplitude > 1.8779; X_n/X_d ratio > 1.685; regression index < -0.4107.
3. No interpretation in relation to sex, age and stage of puberty is necessary for the circadian prolactin secretion rhythm in children.

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