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Corrected 17-Alpha-Hydroxyprogesterone Values Adjusted by a Scoring System for Screening Congenital Adrenal Hyperplasia in Premature Infants

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Abstract. This study investigated the use of corrected 17-alpha-hydroxyprogesterone (17-OHP) values to detect congenital adrenal hyperplasia (CAH) in newborn infants. 17-OHP concentrations in blood spots from 913 neonates were measured using a neonatal screening test. A prematurity index was calculated using a scoring system based on gestational age and birth weight. Blood spot 17-OHP concentrations divided by the sum of prematurity scores were defined as the corrected 17-OHP values. Preterm infants (<30 wk) and low birth weight infants (<1.0 kg) showed 3.9- and 3.8-fold higher blood spot 17-OHP concentrations than normal full term infants. However, no significant differences were observed in the corrected 17-OHP values between the groups. Blood spot 17-OHP levels yielded significant correlations with the prematurity index ($r = 0.42$, $p < 0.05$). Positive results for CAH were obtained in 9.5% (n = 53) and 2.0% (n = 11) of 556 premature infants by the cutoffs of blood spot 17-OHP (>15.0 ng/ml) and corrected 17-OHP values (>13.0 ng/ml), respectively. Of the 53 positive subjects, 39 (73.6%) converted to negative after 1 to 5 mo without treatment. In summary, blood spot 17-OHP levels are influenced by the prematurity of newborns. Use of corrected 17-OHP values provide limited but helpful information in screening for CAH by reducing the rate of false-positive results, especially in premature infants.

Keywords: congenital adrenal hyperplasia, 17-alpha-hydroxyprogesterone in blood spots, 21-hydroxylase deficiency, neonatal screening, premature infants, prematurity index

Introduction

Congenital adrenal hyperplasia (CAH) is a genetic disorder caused by defects in one of several enzymes involved in the synthesis of cortisol in the adrenal glands. More than 90% of cases are caused by 21-hydroxylase deficiency [1]. The clinical symptoms of CAH vary from a simple virilizing type to a severely affected form, such as a life-threatening salt-wasting crisis, depending on the degree of the enzymatic defect [2].

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Measurement of plasma 17-alpha-hydroxyprogesterone (17-OHP) is important for the diagnosis of 21-hydroxylase deficiency. Newborn screening, which is based on the assay of 17-OHP in a dried blood spot on filter paper, is an effective tool for early diagnosis of CAH owing to 21-hydroxylase deficiency [3,4]. However, the 17-OHP test shows a high false-positive rate because of the cross-reactions with steroids other than 17-OHP, especially in preterm infants and critically ill neonates [5,6].

Furthermore, it is difficult to interpret blood spot 17-OHP levels because the 17-OHP values change with gestational age at birth [6]. Conflicting data on the cutoff limits of blood 17-OHP levels in screening for CAH have been reported. Berry et al

[7] proposed the use of two distinct cutoffs with a 10-fold difference in 17-OHP concentrations, such as 20 nmol/L (term infants) and 200 nmol/L (preterm infants), for interpretation of blood spot levels. Ohkubo et al [8] found that the cutoff points of 17-OHP in each gestational age are 91.5 ng/ml (<31 wk), 16.2 ng/ml (32-35 wk), 9.7 ng/ml (36-37 wk), and 8.5 ng/ml (>38 wk). Allen et al [9] reported criteria for 17-OHP levels based on the birth weight of newborns [eg, 165 ng/ml (<1.3 kg), 135 ng/ml (1.3-1.6 kg), 90 ng/ml (1.7-2.2 kg), and 40 ng/ml (>2.2 kg)].

Few studies have investigated integrated cutoff limit for 17-OHP values in blood spots that can be applied to premature infants, irrespective of the stratification of neonates in conjunction with gestational age and birth weight. In the current study, we evaluated a new parameter of corrected 17-OHP values, which are adjusted with a prematurity index using a scoring system.

Materials and Methods

A total of 913 newborns (556 premature infants; 357 mature infants as healthy controls) were investigated by the measurement of blood 17-OHP concentrations, neonatal body weight, and gestational age at birth. Blood specimens were collected on filter paper cards by heel-prick method on 8.5 ± 6.3 days (median, 5 days) after birth, especially focused on premature infants. The 17-OHP levels were determined with the dried blood spots after an extraction procedure, using an enzyme immunoassay (Neonatal 17-OHP kits, Bayer, Tokyo, Japan). The cutoff limit for a positive result in newborn screening was defined as 17-OHP >15 ng/ml on the basis of manufacturer's instructions. All samples with an initial 17-OHP >15 ng/ml in the direct assay were re-analyzed after ether extraction.

The subject cohort was classified into 4 groups based on gestational age and birth weight. Scores (0.5, 1, 1.5, and 2) were assigned to each group in relation to the degree of prematurity, as listed in Table 1, according to the following scheme: infants who were born before the 30 wk of gestation (score 2), 30-33 wk (score 1.5), 34-37 wk (score 1), and >37 wk (score 0.5); neonates with birth weight <1.0 kg (score 2), 1.0-1.49 kg (score 1.5), 1.5-2.5 kg (score 1), and >2.5 kg (score 0.5). To investigate the positive rates in CAH screening test, the 556 premature infants were further stratified into 3 groups; preterm and low birth weight group (n = 397), preterm but normal birth weight group (n = 106), and full term but low birth weight group (n = 53).

Corrected 17-OHP values were calculated using the following formula: the corrected 17-OHP = blood spot 17-OHP concentrations/prematurity index. The prematurity index was obtained from the sum of the scores, which were given to each premature infant in association with the degree of gestational age and birth weight. Positive rates were re-calculated using the corrected 17-OHP level (>13 ng/ml), which was the provisional cutoff point, based on the 99.5 percentile of corrected 17-OHP concentrations in the subject populations.

For the 11 subjects who showed persistent elevation in blood spot 17-OHP levels (>15 ng/ml) in 3 consecutive tests, an ACTH stimulation test was done using a standard dose (250 μg/1.73 m^2) of ACTH (Synacthen; CIBA Lab, Horsham, West Sussex, England) as a bolus iv injection. Blood samples were drawn at 0, 30, and 60 min after the injection. Of the 11 subjects who underwent the ACTH stimulation test, 8 infants who had a 17-OHP/cortisol ratio >0.08 (computed from the data at 30 min after ACTH stimulation) were treated with hydrocortisone, as described previously [10]. Oral hydrocortisone (10-15 mg/m^2/day; Jenapharm GmbH, Germany) was administered for 3 to 9 mo.

Data analysis was conducted using a non-parametric test (the Wilcoxon's rank sum test). Correlation coefficients were calculated by Spearman's method. All p values <0.05 were considered statistically significant.

Results and Discussion

In this study, a new parameter of corrected 17-OHP value was determined using a scoring system, and the parameter was used to screen for CAH in premature infants. The results suggest that the new parameter more accurately reflects enzymatic defects than the uncorrected 17-OHP level, because

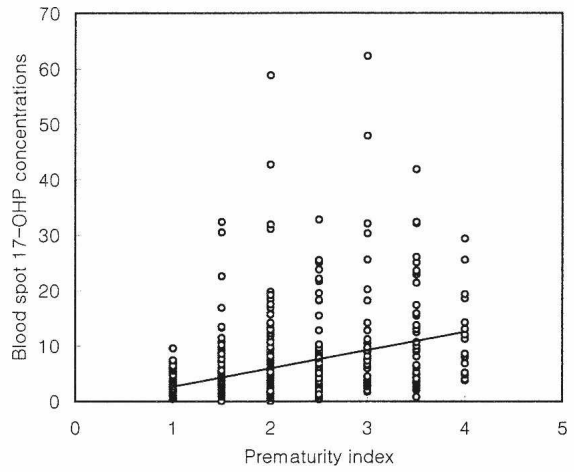

Fig. 1. Scatter plot that shows the correlation between prematurity index (X-axis) and blood spot 17-OHP concentrations (ng/ml, Y-axis) in 913 newborn infants. Prematurity index equals the sum of scores given to each premature infant according to the severity of gestational age and birth weight. The equation of the correlation line is Y = 3.2964X – 0.5831; r = 0.42 (p <0.05).

Table 1. Mean (range; median) values of corrected- and uncorrected-17-OHP concentrations in relation to the prematurity index using a scoring system, as described in the text.

	Subjects (n)	Male:Female	Assigned score	Mean ± SD (range; median)		
				Prematurity index[a]	Uncorrected 17-OHP level (ng/ml)	Corrected 17-OHP level (ng/ml)[b]
Preterm infants (wk)						
< 30	69	37:32	2	3.6 ± 0.3 (3-4; 3.5)	11.7 ± 10.1 (0.8-47.9; 9.1)[c]	3.3 ± 3.0 (0.2-15.9; 2.2)
30 – 33	112	60:52	1.5	2.6 ± 0.2 (2-3.5; 2.5)	8.1 ± 8.3 (0.4-62.3; 5.6)	3.0 ± 2.9 (0.1-20.7; 2.1)
34 – 37	216	102:114	1	2.0 ± 0.2 (1.5-2.5; 2)	5.8 ± 6.5 (0.1-58.8; 3.9)	2.9 ± 3.2 (0.1-29.4; 2.0)
Low birth weight infants (kg)						
< 1.0	19	11:8	2	3.9 ± 0.4 (2.5-4; 4)	11.5 ± 7.3 (1.3-29.6; 8.6)[d]	2.9 ± 1.8 (0.9-7.4; 2.2)
1.0 – 1.49	84	45:39	1.5	3.2 ± 0.4 (2.5-3.5; 3.3)	9.4 ± 10.4 (0.7-60.4; 5.2)	2.9 ± 3.2 (0.2-20.8; 1.6)
1.5 – 2.5	294	154:140	1	2.1 ± 0.3 (1.5-3; 2)	6.7 ± 7.1 (0.1-57.2; 4.6)	3.0 ± 3.2 (0.1-29.4; 2.1)
Controls (normal full term infants)[e]						
> 37 wk; >2.5 kg	357	185:172	0.5[f]	1.0 ± 0.0 (0.5-0.5; 0.5)	3.0 ± 2.3 (0.3-27.1; 2.4)	3.0 ± 2.3 (0.3-27.1; 2.4)

[a] Prematurity index = the sum of the scores assigned according to gestational age and birth weight in premature infants.
[b] Corrected 17-OHP values = Uncorrected 17-OHP levels/prematurity index.
[c,d] Statistically significant ($p < 0.05$) vs preterm infants (34-37 wk) and low birth weight infants (1.5-2.5 kg), respectively.
[e] Controls = infants with full term gestation (>37 wk) and normal birth weight (>2.5 kg).
[f] Score of 0.5 was assigned to each group [ie, gestation > 37wk (score 0.5); birth weight >2.5 kg (score 0.5)].

the value was obtained by adjusting the blood spot 17-OHP concentrations with a prematurity index.

In this study, there were no significant differences in blood 17-OHP levels between the preterm infants of 30-33 wk of gestation and those of 34-37 wk of gestation, nor between the low birth weight infants of 1.0-1.49 kg and those of 1.5-2.5 kg. However, more premature infants with gestational age <30 wk showed 2-fold higher 17-OHP concentrations than those with 34-37 wk of gestation (Table 1). Similarly, very low birth weight infants (<1.0 kg) exhibited a significant increase in 17-OHP levels, compared to those with 1.5-2.5 kg of birth weight (11.5 ± 7.3 vs 6.7 ± 7.1 ng/ml, $p < 0.05$). As shown in Fig. 1, the 17-OHP levels showed a positive correlation with the prematurity index ($r = 0.42$, $p < 0.05$). Our data are in accordance with the results of a previous study, which showed that blood spot 17-OHP concentrations were higher in premature infants than in healthy newborns [11]. These observations suggest that gestational age and birth weight may have some association with blood 17-OHP levels in premature infants, but the impact of these parameters on the 17-OHP level is evident, especially when the birth weight and gestational age are markedly decreased.

Varness et al [12] reported that mean 17-OHP levels were significantly lower in females than males, and that females comprised most of the infants with false-negative results. However, in our study no significant differences were observed in the uncorrected 17-OHP concentrations (6.7 ± 6.1 ng/ml vs 6.5 ± 8.1 ng/ml) and the positive rate for CAH (9.6% vs 9.4%) between male and female infants (data not shown in Table 1). The

Table 2. Mean 17-OHP concentrations, positive rates on the basis of various cutoff limits, and correlation coefficients of 17-OHP levels vs gestational age and birth weight in premature infants.

	Uncorrected 17-OHP concentrations		Positive rates in relation to cutoff limits	
	Mean ± SD (median)	Range	Uncorrected 17-OHP level	Corrected 17-OHP level[a]
Premature infants			>15.0 ng/ml	>13.0 ng/ml
Preterm & low birth weight (n = 397)	7.5 ± 8.0 (4.9)	0.1-62.3	49 (12.3%)	10 (2.5%)
Preterm but normal birth weight (n = 106)	5.4 ± 4.7 (4.5)[b]	0.4-32.4	4 (3.8%)	1 (0.9%)
Full term but low birth weight (n = 53)	3.2 ± 2.1 (2.5)	0.9-10.2	0 (0.0%)	0 (0.0%)
Total (n = 556)	6.7 ± 7.3 (4.4)	0.1-62.3	53 (9.5%)	11 (2.0%)
Relationships with uncorrected 17-OHP levels	r value	p value		
Gestational age (wk)	- 0.31	< 0.05	NA	NA
Birth weight (kg)	- 0.19	< 0.05	NA	NA

[a]Cutoff limit of the corrected 17-OHP values determined on the basis of the 99.5 percentile in the subject populations.
[b] Statistically significant (p <0.05) vs full term but low birth weight, computed by Wilcoxon rank sum test.
Preterm = gestational age <37 wk; full term = gestational age ≥37 wk.
Low birth weight = body weight at birth <2.5 kg; normal birth weight = body weight at birth ≥2.5 kg.
NA = not applicable

inconsistencies may reflect differences in prematurity, growth rate, and postnatal illness of the subject populations, who were recruited in the respective studies.

The cutoff limit of 17-OHP levels for the screening of CAH has been extensively studied. High cutoff values have been used to reduce the false-positive rate. A group of researchers reported a high cutoff point (20 ng/ml) in screening for CAH in premature infants [8]. On the other hand, several investigators proposed that the cutoff level of 17-OHP should be lowered in order to detect more patients [13]. Votava et al [14] found that the false-negative rate was at least one-third in children with the moderate form of CAH using a recommended cut-off limit (30 nmol/L). In addition, Schreiner et al [15] reported that 2.3% of infants with classical CAH were not detected by the usual neonatal 17-OHP screening test. These reports imply that it is difficult to set an ideal cutoff limit that can identify all the affected infants.

In the present study, corrected 17-OHP values were computed in relation to the degree of prematurity. Interestingly, preterm infants (<30 wk) and low birth weight infants (<1.0 kg) revealed no significant differences in the corrected 17-OHP levels compared to the control group, although the 2 groups showed much higher values in uncorrected 17-OHP concentrations than the controls. These findings suggest that falsely elevated 17-OHP concentrations are corrected after amending the blood 17-OHP levels with the prematurity index.

Our scoring system is mainly focused on the premature infants. Because the score of 0.5 is given to each of the infants with full term gestation (>37 wk) and with normal birth weight (>2.5 kg), the sum of scores in the control group (normal full term infants) becomes 1 (0.5 plus 0.5). Thus, in normal newborns, the corrected 17-OHP levels are the same values as the initial blood spot 17-OHP concentrations. Calculating the ratio of 17-OHP levels divided by prematurity scores reduces the influence of perinatal conditions on the newborn screening test, although the parameter does not allow clear distinction between the affected and unaffected neonates.

Among 556 premature infants, 53 (9.5%) were positive in newborn screening by the cutoff limit of uncorrected 17-OHP level (>15.0 ng/ml). However, the positive rate fell to 2.0% (n = 11) when the new parameter of corrected 17-OHP (>13.0 ng/ml) was applied as the cutoff point (Table 2). Follow-up outcomes of the subject populations are summarized in Table 3. Of the 53 infants with a positive result, 39 (73.6%) converted to negative without treatment, after a follow-up period of 1 to 5 mo.

Table 3. Initial data, ACTH stimulation test, and follow-up outcomes in 53 subjects with increase in blood spot 17-OHP levels (>15 ng/ml).

Initial data of 53 subjects	Mean ± SD (median)
Body weight (kg)	1.5 ± 4.3 (1.4)
Gestational age (wk)	29.9 ± 3.0 (29.2)
Initial 17-OHP levels (ng/ml)	30.1 ± 12.8 (27.8)
Duration of therapy (mo)	6.5 ± 2.3 (8.0)
ACTH stimulation test	
After 0 min[a]	
17-OHP levels (ng/ml)	42.6 ± 12.8 (43.9)
Cortisol levels (ng/ml)	132.1 ± 81.5 (124.5)
17-OHP/cortisol ratio	0.39 ± 0.13 (0.38)
After 60 min	
17-OHP levels (ng/ml)	57.5 ± 17.5 (57.5)
Cortisol levels (ng/ml)	481.2 ± 64.3 (469.3)
17-OHP/cortisol ratio	0.12 ± 0.03 (0.11)
Treatment with oral hydrocortisone[b]	
17-OHP levels after 1 mo (ng/ml)[c]	3.2 ± 3.5 (1.9)
Follow-up outcomes	No. of subjects (n)
Negative conversion without treatment	39 (73.6%)
After 1 mo	32 (59.3%)
After 2 mo	3 (5.6%)
After 3 mo	1 (1.9%)
After 5 mo	3 (5.6%)
Lost to follow-up	5 (11.1%)
Death	1 (1.9%)

[a] ACTH stimulation tests were performed in 11 subjects showing a persistent elevation in 17-OHP levels.
[b] Oral hydrocortisone was administrated in 8 infants with 17-OHP/cortisol ratio >0.08.
[c] 17-OHP levels were measured at 1 mo after the cessation of hydrocortisone therapy.

These results indicate that a considerable number of positive subjects become normalized in 17-OHP level as they grow older. Our data also suggest that the maturation of adrenal gland may be delayed in some infants and the production of CAH-related enzymes may be retarded. It appears that the cutoff limit of uncorrected 17-OHP level lacks sufficient discriminating ability in newborn screening for CAH. Application of the new index of corrected 17-OHP value diminishes the false-positive rate in premature infants, which appears to be influenced by gestational age or birth weight.

To investigate the parameters that are closely associated with 17-OHP levels, premature infants were evaluated on the basis of gestational age and birth weight. The preterm but normal birth weight infants had significantly higher 17-OHP levels than the full term but low birth weight infants (5.4 ± 4.7 ng/ml vs 3.2 ± 2.1 ng/ml, $p < 0.05$). Among the 106 preterm but normal birth weight infants, 4 (3.8%) were positive for uncorrected 17-OHP test, but none was positive in the full term but low birth weight infants. Uncorrected 17-OHP levels were more strongly correlated with gestational age ($r = -0.31$, $p < 0.05$) than birth weight ($r = -0.19$, $p < 0.05$) in premature infants.

These results suggest that gestational age may be more critical than body weight in CAH-related enzyme production. Our data agree with the results of prior studies, which showed that gestational age is a better predictor of 17-OHP in newborns than birth weight [16], and that adrenal cortical function in preterm infants is closely related to the duration of gestation [17].

Early recognition and treatment of CAH prevent the male sex assignment in virilized females, which is caused by androgen overproduction, but the most important rationale of treatment is prevention of a life-threatening salt-wasting crisis [18]. In the current study, oral hydrocortisone was administered to 8 newborns who had an elevated 17-OHP/cortisol ratio after an ACTH stimulation test. The raised 17-OHP levels (42.6 ± 12.8 ng/ml) were decreased to 3.2 ± 3.5 ng/ml by the treatment, which were measured after 1 mo of therapy. During the follow-up period, there were no subjects who showed CAH-related symptoms or significant re-elevation of the 17-OHP level.

In our study, the absence of relapse after discontinuation of therapy may be discordant with the notion that the treated group have an inherited disorder caused by a genetic defect. The possibility that the subjects did not have true CAH, but a reactive change of uncertain significance, can be postulated. These findings suggest that careful attention should be paid to the interpretation of 17-OHP levels, and treatment should be based on cumulative data at least for 3 mo. Our observations are consistent with the study of Santos et al [19], which concluded that decisions regarding therapy should be made on 17-OHP interpretation in relation to the patient's clinical history and growth parameters.

In the present study, the cutoff point (13 ng/ml) of corrected 17-OHP levels corresponds to 32.8 ng/ml of uncorrected 17-OHP concentration, which was the median value of blood spot 17-OHP levels in the 53 subjects with a positive result. In this study we could not test the predictive value of the cutoff limit of corrected 17-OHP because there were insufficient numbers of confirmed CAH patients in our subject population. The present study measured blood spot 17-OHP levels only in suspected CAH infants. Further study is needed regarding the potential application of the corrected 17-OHP value in infants with the severely affected form of CAH.

In conclusion, this study tested a new parameter, the corrected 17-OHP level, to screen for CAH, especially in premature infants. This method may help to reduce the high false-positive rate, avoid unnecessary follow-up tests, and prevent misdiagnosis and misjudgement of treatment.

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