Longitudinal measurements of 17 α -hydroxyprogesterone in premature infants during the first three months of life

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Abstract

Aims—To determine normal concentrations of 17 α -hydroxyprogesterone (17OHP) for premature infants.

Methods—17OHP was measured in 66 consecutive premature infants once a week during the first month, and once every two weeks thereafter, until the age of 3 months. The 17OHP values in 100 full term healthy neonates on the third day of life served as controls. Blood was sampled on filter paper using a neonatal radioimmunoassay kit. Findings were correlated with gestational age, birthweight, mode of delivery, Apgar scores, presence of respiratory distress syndrome and intake of maternal steroids.

Results—Mean 17OHP was raised at 7 days of age (138.9, 46.3, 53.3, 29.9 nmol/l, respectively, for infants whose gestational age was under 29 weeks, 29 to 30 weeks, 31 to 32 weeks, and 33 weeks and above). It fell sharply in the first two weeks after which it gradually decreased further, reaching 32.7, 23.6, 16.9, and 13.0 nmol/l, respectively, by the age of 90 days. The mean (SEM) 17OHP concentration in full term infants on day 3 of life was 17.8 (8.9) nmol/l. These values were independent of the presence and severity of respiratory distress syndrome and of prenatal maternal steroids.

Conclusions—The increased 17OHP concentrations found at birth fell to those found in term infants during the first three months of life in infants over 31 weeks of gestation. Postconceptional age is the most important factor determining 17OHP concentration.

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Keywords: 17 α -hydroxyprogesterone; postconceptional age; congenital adrenal hyperplasia; steroids

Congenital adrenal hyperplasia (CAH) is a family of recessive inherited disorders of adrenal steroid hormone origin, caused in 90–95% of cases, by 21-hydroxylase deficiency.¹ CAH is suspected in newborn girls with ambiguous genitalia, phenotypic boys with bilateral cryptorchidism, older boys with progressive virilisation and in neonates with acute adrenal insufficiency.^{2–4} The diagnosis is readily confirmed by increased 17 α -hydroxyprogesterone (17OHP) concentrations.

Over the past two decades neonatal (age 2–5 days) screening programmes for high 17OHP

values using heel blood spots have been instituted in several regions and countries.^{1 5–7} In Israel a nationwide random screening programme revealed a high incidence of CAH in the Arab population, which prompted a second programme for neonates in northern Israel.⁸ One of the major problems encountered in our programme and in others is the high incidence of false positive results in preterm infants.^{2–5 8–13}

This study aimed to determine normal concentrations of 17OHP for premature infants during the first three months of life and to correlate these with postconceptional age, birthweight, incidence and severity of respiratory distress syndrome, and antenatal use of steroids.

Methods

The study was conducted in the neonatal intensive care unit of the Chaim Sheba Medical Center. Sixty six consecutive premature infants (gestational age <35 weeks, birthweight <2000 g), born between January and August 1994, were included. One hundred term healthy infants (gestational age >37 weeks, birthweight >2500 g) served as controls. The study was approved by the hospital's human rights committee, and informed consent was obtained from the parents of each participant. The clinical characteristics of the patients are shown in table 1. 17OHP was correlated with sex, Apgar score, mode of delivery (vaginal or caesarean) the need for blood transfusions, presence of neonatal sepsis, incidence and severity of respiratory distress syndrome—mild, need for oxygen; moderate, need for mechanical ventilation for up to 5 days; severe, need for mechanical ventilation > 5 days—or prenatal treatment with dexamethasone for fetal lung immaturity.

In the preterm group blood was sampled on filter paper during the first 24 hours, then once a week during the first 28 days, and thereafter every two weeks until 60 days of age, and again at 90 days. In the term group blood samples were obtained on the third day of life. 17OHP concentrations were measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA) in the National Unit for Congenital Hypothyroidism and Screening Program for 21-hydroxylase deficiency.⁸

Blood spot 17-OHP concentrations were determined using a solid phase ¹²⁵I radioimmunoassay with the Coat-A-Count Neonatal 17-alpha-OH Progesterone kit (Diagnostic Products Corporation, Los Angeles, CA).

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Table 1 Clinical characteristics of patients (n=66)

	Full term (n=100)	Preterm (n=66)
Gestational age (weeks) (mean (SD))	39.2 (2.7)	31.8 (2.1)
Birth weight (g) (mean (SD))	3,100 (448)	1488 (291)
Male/female	47%/53%	45.5%/54.5%
Type of delivery:vaginal/caesarean section	92%/8%	28%/72%
Apgar score 1 minute (mean (SD))	9	7.4 (2.4)
Apgar score 5 minutes (mean (SD))	10	8.9 (1.2)
Single/twin/triplet	100% singleton	50%/28.8%/21.2%
Maternal steroids (%)	None	41 (60.2%)
Respiratory distress syndrome	None	24 (35%) (mild: 6, moderate: 11, severe: 7)
Blood transfusions	None	10 (15.2%)
Sepsis	None	3 (4.4%)

The procedure is based on a polyclonal (rabbit) antibody and designed for use with blood spot samples. ^{125}I -labelled 17OHP competes for a fixed time with 17OHP in the patient sample for antibody sites. As the antibody is immobilised to the wall of a tube, decanting suffices to terminate the competition and to isolate the antibody bound fraction of the radiolabelled 17OHP. Counting the tube in a gamma counter then yields a number which converts, by way of a calibration curve, to a measure of the 17OHP in the patient sample.

The sensitivity of the 17OHP assay is 0.8 nmol/l and the intra- and interassay coefficients of variation (CV) are 6% and 0%, respectively. Cross reactivity with other naturally occurring steroids or therapeutic drugs is extremely low, maximally to 17OHP (3.5%) and to 11-deoxycortisol (0.8%).

Statistical analysis was performed using BMDP Statistical Software.¹⁴ One and two way analysis of variance and covariance with repeated measures, Fisher's exact test, Pearson's χ^2 test, paired *t* test and regression analysis were used, as appropriate. Correlations were performed using Student's linear regression analysis.

Results

Mean 17OHP concentrations by day 7 of life were higher in the premature infants than in full term infants on day 3 of life ($p<0.01$). Concentrations sharply declined in the first two weeks, and then continued to decrease more gradually. By the age of 3 months, the average 17OHP concentrations for preterm infants over 29 weeks decreased to those of full term infants at the age of 3 days. However, for

Figure 1 17OHP concentrations according to postconceptional age.

Table 2 17OHP concentrations (nmol/l) in preterm infants according to birthweight groups

Age (days)	Birthweight (g) Mean (SD)		
	500–1000 n=6	1001–1500 n=24	1501–2000 n=36
1	249.6 (143.8)	101.9 (55.5)	86.4 (36.7)
7	171.3 (71.4)	51.6 (35.1)	47.4 (28.0)
14	67.0 (33.0)	43.2 (23.7)	38.7 (13.5)
21	44.2 (13.1)	37.5 (15.4)	42.5 (18.5)
28	41.6 (13.3)	46.1 (18.7)	45.7 (21.0)
45	29.6 (6.9)	40.4 (12.4)	33.1 (17.0)
60	34.4 (30.2)	28.8 (8.2)	22.5 (14.0)
90	31.8 (2.1)	20.9 (12.5)	15.9 (6.3)

infants below 29 weeks, the concentration at 3 months was almost double that of full term infants ($p<0.05$). Only those who were born at more than 33 weeks of gestation achieved term values by 60 days of age. During the first two weeks of life, 17OHP concentration was inversely correlated with postconceptional age and birthweight. Analysis of variance and covariance was significant for both factors, but stronger for postconceptional age ($p<0.0001$) (fig 1) than for birthweight (table 2) ($p<0.004$). The normalisation of 17OHP concentrations was slower in the smaller and younger infants (500–1000 g; 26–28 weeks, respectively). Analysis of variance and covariance showed no relation between 17OHP concentrations and sex, Apgar score, mode of delivery, need for blood transfusions, presence of neonatal sepsis, incidence and severity of respiratory distress syndrome, or prenatal treatment with dexamethasone for fetal lung immaturity. Regression analysis revealed a significant influence for both birthweight and postconceptional age on 17OHP concentrations ($R^2=0.058$, $F=11.9$, $p<0.00001$).

The higher rate of respiratory distress syndrome in the young and low birthweight premature infants gave the impression that these infants have higher 17OHP concentrations. However, analysis of variance and covariance showed that it was gestational age rather than the lung disorder which was the significant factor.

Discussion

Although pilot programmes for infant screening for high 17OHP concentrations began more than 15 years ago, only 23 are used today in 10 countries. This is partly due to the high financial outlay required, but also to the psychological cost of the high rate of false positive results,¹⁵ particularly in preterm, small for gestational age, and sick infants under stress.^{5 11} The latter may be attributed to the following: differences in the activity of enzymes in the glucocorticoid pathway which may be developmentally regulated to increase with advancing postconceptional age; decreased metabolic clearance of 17OHP due to immature hepatic function; physiological pituitary adrenal stress response; and cross reaction with conjugated steroids in the premature neonate's serum.^{4 10 12} For these reasons, higher recall values for preterm infants have since been established in the various screening programmes (table 3).

Table 3 17 α -hydroxyprogesterone concentrations in preterm infants: review of published findings

Author Year	No of preterm infants	Time of collection	17OHP (nmol/l)	Remarks
Cacciari <i>et al</i> ¹⁷ 1983	Total 41 299 preterm 3935	Days 1–15	Peak 13 pg/disk range 13–5 pg/disk	Infant maturity and day of collection affect values
Wallace <i>et al</i> ⁸ 1985	376 (direct method) 118 (extraction method)	Second week after birth	Direct: median 31 nmol/l extraction: median 13.5 range 0–75 nmol/l	Results were lower in extraction assay
Berry <i>et al</i> ¹² 1986	32	Days 3,8,14	Peak 130 nmol/l (day=3) range 20–80 nmol/l	Upper limit: term infants—20 nmol/l preterm—200 nmol/l
Laresson <i>et al</i> ¹⁰ 1988	Total 22 400 (preterm + full term)	After 72 h (median 5 days)	n=201–>100 nmol/l n=13–>400 nmol/l	Correlation with gestational age
Thompson <i>et al</i> ⁹ 1989	89 (<2500 g)	Days 1–24	n=11–>30 nmol/l	Day of collection did not influence results
Lee <i>et al</i> ¹¹ 1989	22 (13 healthy, 9 sick)	Days 2–5	Healthy 28.5 (6.2) nmol/l mean (SD) sick 42.2 (7.2) nmol/l	Concentrations are higher in sick preterm infants
Knudtzon <i>et al</i> ⁷ 1991	26	Days 3–17 (mean 7.7)	Mean (SD) 53.3 (6.3) nmol/l	Negative correlation with gestational age and birth weight
Ohkubo <i>et al</i> ¹³ 1992	836	Days 3 to 96	<29 w—2.0 (1.39) nmol/l 30–34 w—1.3 (0.85) nmol/l 35–36 w—0.82 (0.85) nmol/l (log values) Mean (SD)=11.4 (11.1) nmol/l	ELISA
Hingre <i>et al</i> ¹⁸ 1994	25 (<1250 g)	Day 4	Mean (SD)=11.4 (11.1) nmol/l range 1.4–82.5 nmol/l	High concentrations in very low birthweight infants
Al Saedi <i>et al</i> ⁴ 1995	39 healthy <31 weeks	Once a week until age 37 weeks	Mean (SD)=11.4 (11.1) nmol/l range 1.4–82.5 nmol/l	Predicted 17OHP concentrations: 9.4–13—initial 5.2–5.3—35 to 56 days of age
Allen <i>et al</i> ¹⁵ 1997	149 684	At time of discharge	Multitiered reporting scheme: 1299 g—499.3 nmol/l 1300–1699 g—408.5 nmol/l 1700–2200 g—272.3 nmol/l >2200 g—121.0 nmol/l	Weight adjusted criteria reduced the false positive results without diminishing sensitivity
Linder <i>et al</i> 1999 (present study)	66	Once a week during the first month and then every two weeks until age 3 months	17OHP (nmol/l) Day <29 29–30 31–32 >33 week week week week 1 179.1 98.3 99.6 78.1 7 138.9 46.3 53.3 29.9 14 63.2 40.2 42.9 29.7 21 45.1 40.2 44.3 31.2 28 42.1 48.7 48.1 32.1 45 37.5 35.4 35.4 29.7 60 35.0 26.9 25.1 17.8 90 32.7 23.6 16.9 13.0	17OHP concentrations decline to full term concentrations during the first three months of life. Birthweight and gestational age affect level

Our results confirm that normal 17OHP values for preterm infants are inversely correlated with postconceptional age and birthweight^{11–13 16}

Contrary to Thompson *et al*,³ who reported that age at the time of specimen collection had no effect on 17OHP concentrations in infants weighing under 2500 g at birth, we found that they sharply declined in the first two weeks of life and thereafter gradually reached full term values within the first three months of life. This phenomenon was accentuated in very young and low birthweight infants.

Al Saedi *et al*⁴ were the first to describe sequential sampling of 17OHP concentrations in preterm infants, performed weekly until 37 weeks of age. However, their mean concentrations were lower than those found in this study. Normal 17OHP concentrations vary considerably, as noted in the various screening programmes; this reflects differences in the assay used (RIA, ELISA, FIA), antibody specificity, and the thickness of the blood spot.⁵ Mean 17OHP concentrations were not affected by the incidence of caesarean section, low Apgar scores, incidence and severity of respiratory distress syndrome, or maternal use of steroids. Gestational age had a greater effect on 17OHP concentrations than birthweight. This contrasts with the findings of Cacciari *et al*,¹⁶ who found that birthweight was more significant than gestational age in determining 17OHP concentration in infants weighing under 2500 g.

Recently, Allen *et al*¹⁵ suggested a four tiered weight adjusted threshold for 17OHP. This reduced the number of false positive results, particularly among low birthweight infants, without diminishing the sensitivity of the test. Birthweight was chosen for categorisation because it was thought that the birthweight data supplied on the newborn screening form were more reliable and accurate than those on gestational age. Their criteria, however, did not take into account the day of specimen collection, an important variable in low birthweight babies.

In conclusion, increased 17OHP concentrations in premature infants may lead to a misdiagnosis of congenital adrenal hyperplasia. Notably, 17OHP concentrations in premature infants are independent of maternal glucocorticoid treatment, and the incidence and severity of respiratory distress syndrome, and will normally reach full term values within the first three months of life.

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