

MONITORING CONGENITAL ADRENAL HYPERPLASIA USING BLOOD SPOT 17 - HYDROXYPROGESTERONE ASSAY

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Abstract. Blood spots taken by finger prick collected on filter paper cards can provide an option to venous blood extraction in monitoring 17-OHP levels in children with Congenital Adrenal Hyperplasia (CAH). This study was done to evaluate the usefulness of blood spot 17-OHP in monitoring disease control in pre-pubertal children with CAH, to correlate it with simultaneously extracted venous 17-OHP levels, and to compare blood spot levels of children with CAH with that of normal non-virilized children. Nine pre-pubertal children with CAH (1 male; 8 females) were enrolled in the study. Age, sex, growth velocity, height age and bone age were determined. Simultaneous venous and blood spot specimens were taken between 0800 and 0900 hours. Nine pre-pubertal, age- and sex-matched normal non-virilized children served as controls. COAT-A-COUNT® was used to measure venous 17-OHP levels, and AutoDELFIATM Neonatal 17 α -OH-progesterone was employed for blood spot specimens. Mean age of patients with CAH was 42.78 months (SD= 21.45214). Four had simple virilizing form and five were salt-losers. Venous 17-OHP levels ranged from 7.5 to 800nmol/l. Blood spot 17-OHP levels ranged from \leq 0.5000nmol/l to 355.5nmol/l. There was a strong positive correlation between the venous and blood spot determination, with a correlation coefficient $\Gamma = 0.947$ ($p < 0.001$). All of the children in the control group had a blood spot 17-OHP level \leq 0.5000nmol/l. Taking blood spot 17-hydroxyprogesterone levels is a simple, acceptable, convenient, and less costly alternative to venous 17-OHP determination in monitoring treatment response of children with CAH. The decision to make treatment modification, however, should be made on random blood spot 17-OHP interpretation in conjunction with clinical history and evaluation of growth parameters.

INTRODUCTION

Congenital adrenal hyperplasia (CAH) due to classic 21-hydroxylase deficiency has a worldwide incidence of 1:10,000 to 1:15,000 live births. Together with 11- β -hydroxylase deficiency, they account for more than 90 to 95% of cases.

Standard treatment of 21-hydroxylase deficiency includes glucocorticoid administration, with or without mineralocorticoid and NaCl supplementation. The protocol for monitoring treatment, however, varies. Serum levels of 17-hydroxyprogesterone (17-OHP), androstenedione, testosterone, and renin, measured preferably between 7:30 and 8:30 AM, either before or after taking the morning medication, usually provide adequate indices of control (AAP Technical Report, 2000). Observing for signs of cortisol or androgen excess, growth, pubertal and osseous maturation is equally important.

In our practice, serum 17-OHP level is regularly taken to ascertain the need to revise steroid dose or dose schedule. The measurement of serum 17-OHP in a filter paper capillary blood specimen is used mainly to screen newborn infants for 21-hydroxylase deficiency. However, several authors have also found this technique useful in monitoring treatment response of these patients (Bode *et al*, 1999; Appan *et al*, 1989; Young *et al*, 1988; Riordan *et al*, 1984; Schwartz, 1999; Erhardt *et al*, 2000; Shimon *et al*, 1995).

Current guidelines in the interpretation of venous or blood spot 17-OHP values vary. Thus, under- or over-treatment of children with CAH may be difficult to assess unless clinical changes are already apparent. In this study, treatment response of children with CAH was evaluated by correlating clinical parameters with blood spot 17-OHP levels, using blood spot 17-OHP of normal children as an arbitrary normal range.

OBJECTIVES

The objective of this study is to evaluate the usefulness of blood spot 17-OHP in monitoring disease control of children with CAH. Specifically, it aims to: (1) correlate capillary blood spot 17-OHP with the standard venous blood assay in pre-pubertal children with congenital adrenal hyperplasia, and (2) compare blood spot 17-OHP of children with CAH with that of age and sex-matched normal non-virilized children

MATERIALS AND METHODS

Subjects

Nine pre-pubertal children with congenital adrenal hyperplasia due to 21-hydroxylase deficiency were included in the study. The diagnosis of CAH was based on classical signs and symptoms of androgen excess with or without symptoms of insufficiency of cortisol and/or mineralocorticoid, confirmed with an elevated serum 17-OHP. Salt losers were patients with low serum sodium accompanied by elevated serum potassium levels at the time of diagnosis. Patients on adrenal crisis were not included in the study. Age and sex-matched normal unaffected healthy children were recruited from the well child clinic to serve as controls. Chronologic age, sex, height in cm, weight in kg and blood pressure were taken. The current treatment regimen including type, dose, and dose schedule of the steroid/s, growth velocity, height age, and bone age were included in the data collection.

Specimen collection

All blood samples were taken between 0800 and 0900 hours for both subjects and controls. Patients with CAH were advised to withhold their morning dose prior to specimen collection. However, Patients 6 and 7 were given their hydrocortisone dose 1 hour prior to sampling. Patients 2 and 3, on the other hand, had not been taking steroids for two weeks prior to testing.

a. Sample collection for serum radioimmunoassay (RIA)

A 3-cc venous blood sample was collected in a plain test tube, and allowed to clot and retract. Specimens were centrifuged, and sera were collected in microtubes, then stored in a freezer (-20°C) for a maximum of two weeks until completion of collection from all nine patients. The level of 17-OHP was obtained using COAT-A-COUNT® RIA techniques. All samples were assayed simultaneously by a single technician blinded to the disease status of the patients.

b. Sample collection for blood spot fluoroimmunoassay

A finger prick using a standard blood lancet was done on all subjects and controls, except on Patient 7, who had a heel prick. Blood spots were collected on Guthrie filter paper cards. Specimens were allowed to air dry, then stored at room temperature for a maximum of two weeks until samples from all subjects have been taken. Fluoroimmunoassay of 17-OHP was done using AutoDELFIATM Neonatal 17 α -OH-progesterone kit. A single technician performed the test and was blinded to the identity of the subjects.

Statistical analysis

Descriptive statistics for patient characteristics included computation of mean and standard deviation, and percentages.

Pearson's coefficient of correlation was used to measure strength of correlation between venous and blood spot 17-hydroxyprogesterone assays. Statistically significant correlation was set at p value of <0.05.

DEFINITION OF TERMS

1. Chronologic age was the time interval from date of birth to specimen collection.
2. Sex was defined by karyotype.
3. Growth velocity was height (in cm.) gained in the preceding 3 to 6 months. It was classified as normal if it was appropriate for age.
4. Height age was the age at which the subject's height fell into the 50th percentile as extrapolated from the NCHS growth charts. Normal was height age within 6 months of chronologic age.
5. Bone ages were interpreted according to Greulich and Pyle standards. Results were classified as appropriate if the bone age was within 6 months of the chronologic age.

RESULTS

There were 9 females and 1 male. All were pre-pubertal and had no overt signs of Cushing Syndrome. Ages ranged from 5 to 86 months, with a mean of 42.78 months (SD= 21.45). Four were simple virilizing (44.4%) and 5 were salt-losing (56.4%). None of the patients were hypertensive. Steroid doses ranged from 9.4 to 30.7 mg of cortisol/m²/day, with a mean of 15.72 (SD= 6.34). Patients 7 and 8 were receiving hydrocortisone and fludrocortisone, Patient 5 was taking NaCl tablets with prednisone, while the others were taking prednisone alone at the time of the study. A summary of these data is shown on Table 1.

Biochemical evaluation

Venous 17-OHP levels ranged from 7.5 to 800nmol/l. Blood spot 17-OHP levels varied from 0.5000 to 411.7nmol/l. Mean blood spot 17-OHP was 145.13nmol/l (SD = 174.62) and mean venous 17-OHP was 359.39nmol/l (SD = 338.95). Blood spot values were consistently lower than venous 17-OHP values. Table 1 lists values of simultaneous venous and blood spot 17-OHP of the nine children with CAH.

A scatter plot diagram in Fig 1 shows the strong positive correlation between the venous and blood spot 17-OHP levels $G = 0.947$ which was statistically significant at p value of < 0.001 .

When compared with published normal values for serum 17-OHP level for normal pre-pubertal children (8), all of the subjects with CAH had elevated venous 17-OHP levels. Since, blood spot norms for 17-OHP in pre-pubertal children have not been established, blood spots from nine normal, non-virilized children of similar age and sex were taken and assayed for 17-OHP for control comparison with patients' levels. All had a blood spot 17-OHP level of 0.50nmol/l, the detection limit for the assay. Thus, the normal 17-OHP levels for non-virilized pre-pubertal children was taken to be ≤ 0.5000 nmol/l. Therefore, all subjects with CAH, except Patient 1, had elevated blood spot 17-OHP.

To evaluate the usefulness of blood spot 17-OHP in monitoring treatment response, growth velocity, height age and bone age were taken from the nine patients to

corroborate degree of control implied by blood spot 17-OHP levels. Eight of the 9 subjects with CAH had elevated blood spot 17-OHP and the proportion of children with clinical signs of androgen excess were evaluated in this group. Four of the 8 had increased growth velocity (50%), 1 (12.5%) had increased height age, and 3 (37.5%) had advanced bone age. Conversely, three of the 8 (37.5%) still had normal growth velocity, three (37.5%) had normal height age, and five (62.5%) had appropriate bone age despite elevated levels. The only patient with a blood spot 17-OHP level ≤ 0.5000 nmol/l (Patient 1) had decreased growth velocity, decreased height age, and appropriate bone age, which are signs of glucocorticoid excess.

DISCUSSION

The goal of therapy in children with CAH is to prevent adrenal insufficiency and to suppress excessive androgen production with exogenous cortisol while ensuring adequate growth and appropriate bone maturation. Adult height of patients with 21-hydroxylase deficiency is often within 1SD of the target height, but early diagnosis and good compliance improve final height outcome (Eugster *et al*, 2001). Since the desired end result of treatment is normal growth, some authors believe that the best method of monitoring treatment is to concentrate on clinical parameters rather than on biochemical results (Appan *et al*, 1989). These include measurement of growth velocity and skeletal maturation (bone age), signs of hypercortisolism (striae, weight gain, hypertension), and pattern of menses in postpubertal women. Of these, a decrease in growth

Table 1. Patient Characteristics, Venous and Blood Spot 17-hydroxyprogesterone.

Patient	Sex	Age (months)	Form of CAH Pressure	Blood	Steroid dose (mg cortisol/m ² /day)	Venous 17-OHP	Blood spot 17-OHP
1	F	48	salt losing	normal	17.8	7.5	0.5000
2	F	33	simple virilizing	normal	11.76	740	349.8
3	F	48	simple virilizing	normal	15.2	800	355.5
4	F	83	simple virilizing	normal	16.3	730	411.7
5	M	49	simple virilizing	normal	30.7	28	5.8
6	F	5	salt losing	normal	9.8	55	6.4
7	F	49	salt losing	normal	9.4	34	2.76
8	F	23	salt losing	normal	16	380	80.8
9	F	47	salt losing	normal	14.5	460	93
Mean		42.78			15.72		
SD		21.454			6.34		

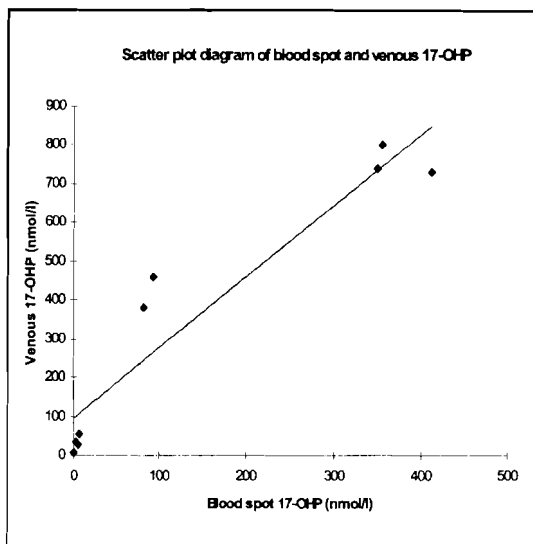


Fig 1. Scatter plot diagram of blood spot and venous 17-OHP assay.

velocity is the most sensitive clinical indicator of corticosteroid excess. Biochemical markers, on the other hand, are as important in regulating steroid doses because clinical changes can only be measured after an elapsed period of time. Thus, treatment response using these clinical changes can only be assessed in retrospect (Hughes, 1988). Biochemical parameters such as serial or random plasma 17-OHP measurements, however, provide an insight to the current degree of adrenal suppression.

As in previous studies done separately by Bode *et al* in 1999 and Shimon *et al* in 1995, this study showed that blood spot 17-OHP levels strongly correlated with venous blood levels. Erhardt *et al* in 2000 compared serial blood spot 17-OHP with 24-hour urinary steroid excretion and found strong correlation ($r=0.839$ at $p<0.001$) as well. Therefore, blood spot 17-OHP is a useful and a simple alternative in monitoring treatment response of children with 21- hydroxylase deficiency.

Single random determinations of plasma 17-OHP are difficult to interpret because of combined influence of stress, intrinsic circadian rhythm, and the timing of previous glucocorticoid medication on hormone concentration (Appan *et al*, 1989). Elevated levels may indicate inadequate dose of glucocorticoid or non-compliance; extremely low levels may indicate either good control or excessive glucocorticoid replacement. The need to modify present treatment, therefore, cannot be based

solely on a single 17-OHP determination, and levels need to be correlated with other clinical parameters.

In this study, a normal random blood spot 17-OHP level (≤ 0.5000 nmol/l) appeared to be associated with signs of glucocorticoid over-treatment. In the evaluation of serial blood spot 17-OHP levels, Bode *et al*, suggested maintaining blood spot 17-OHP levels at <10 nmol/l for adequate adrenal suppression (Bode *et al*, 1999). Silva *et al*, on the other hand, found that growth velocity and serum 17-OHP concentrations are positively correlated, and that optimal growth velocity occurred at average plasma 17-OHP around 21nmol/l. They further suggested that normal serum 17-OHP levels should not be considered a treatment goal, but an indication of excessive corticosteroid treatment in these patients (Silva *et al*, 1997).

CAH patients with elevated random 17-OHP blood spot levels in this study seemed more likely to have increased growth velocity, a sign of androgen excess. However, not all patients with random blood spot 17-OHP levels > 0.5000 nmol/l had signs of chronic androgen excess. Although inadequate steroid dose is the most likely cause of elevation in the 17-OHP level, other causes of transient rises in 17-OHP such as missed doses, stress, or infection must be considered and ruled out before making the necessary dose adjustments. Daily serum 17-OHP profiles for extended periods may confirm consistently elevated values, and home monitoring of blood spot 17-OHP may be most helpful in these instances.

CONCLUSIONS

Blood spot 17-OHP levels correlated well with simultaneous venous 17-OHP levels in pre-pubertal children with congenital adrenal hyperplasia. It is, therefore, a simple, convenient, and less costly alternative to venous 17-OHP determination in monitoring treatment response of children with CAH. Blood spot 17-OHP levels of normal non-virilized pre-pubertal children were consistently less than or equal to 0.5000 nmol/l. Single random blood spot 17-OHP levels are best correlated with clinical history and growth parameters for appropriate interpretation.

RECOMMENDATIONS

Serum 17-OHP profiles over extended periods can be established using 17-OHP blood spot monitoring. This may give a helpful insight into the 24-hour control of the disease. Likewise, through analyzing growth patterns with serial hormonal determinations, a normal range of

serum 17-OHP for different ages can be established for patients with CAH.

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