Skeletal Maturation in Children with Cushing Syndrome Is Not Consistently Delayed: The Role of Corticotropin, Obesity, and Steroid Hormones, and the Effect of Surgical Cure

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Objective To assess skeletal maturity by measuring bone age (BA) in children with Cushing syndrome (CS) before and 1-year after transsphenoidal surgery or adrenalectomy, and to correlate BA with hormone levels and other measurements.

Study design This case series conducted at the National Institutes of Health Clinical Center included 93 children with Cushing disease (CD) (43 females; mean age, 12.3 ± 2.9 years) and 31 children with adrenocorticotropic hormone–independent CS (AICS) (22 females, mean age 10.3 ± 4.5 years). BA was obtained before surgery and at follow-up. Outcome measures were comparison of BA in CD vs AICS and analysis of the effects of hypercortisolism, insulin excess, body mass index, and androgen excess on BA.

Results Twenty-six of the 124 children (21.0%) had advanced BA, compared with the expected general population prevalence of 2.5% ($P < .0001$). Only 4 of 124 (3.2%) had delayed BA. The majority of children (76%) had normal BA. The average BA *z*-score was similar in the children with CD and those with AICS (0.6 ± 1.4 vs 0.5 ± 1.8; $P = .8865$). Body mass index SDS and normalized values of dehydroepiandrosterone, dehydroepiandrosterone sulfate, androsteonedione, estradiol, and testosterone were all significantly higher in the children with advanced BA vs those with normal or delayed BA. Fifty-nine children who remained in remission from CD had follow-up BA 1.2 ± 0.3 years after transsphenoidal surgery, demonstrating decreased BA *z*-score (1.0 ± 1.6 vs 0.3 ± 1.4; $P < .0001$).

Conclusion Contrary to common belief, endogenous CS in children appears to be associated with normal or even advanced skeletal maturation. When present, BA advancement in CS is related to obesity, insulin resistance, and elevated adrenal androgen levels and aromatization. This finding may have significant implications for treatment decisions and final height predictions in these children. *(J Pediatr 2014;164:801-6)*.

Although growth failure is a hallmark of Cushing syndrome (CS) in the pediatric population,[1-4] only a limited number of reports describe the status of skeletal maturation at the time of diagnosis of Cushing disease (CD), and none describe bone age (BA) in adrenocorticotropic hormone (ACTH)-independent CS (AICS). Glucocorticoids impair somatic growth by directly inhibiting the development of epiphyseal cartilage in growing long bones.[5] Androgens induce epiphyseal closure via aromatization to estrogens.[6] CD is associated with elevated ACTH, excessive virilization, and increased adrenal androgens.[7]

Prolonged exposure to exogenous corticosteroids is associated with growth inhibition and delayed skeletal maturation. A 1965 study of 36 children with adrenocortical virilism, hypopituitarism, Addison disease, and allergy demonstrated that administration of hydrocortisone 35-50 mg/m^2/day tended to reduce rates of growth and skeletal maturation below normal.[8] Skeletal maturation is delayed in children with chronic disease who receive long-term exogenous glucocorticoid therapy.[9] The effects of corticosteroid treatment on growth and skeletal maturation have been evaluated in children with severe asthma; however, poor growth, and pubertal delay owing to underlying disease might have independent effects on bone maturation. Interestingly, suppression of BA advancement is reportedly more profound in boys than girls with asthma exposed to glucocorticoids.[10] Inhibition of growth and skeletal maturation by exogenous steroids has been shown to be dose-dependent.[11] Additional evidence of the effect of steroids on inhibition of skeletal maturation comes from the observation that after suspension of treatment, bone maturation of children on steroid therapy was equivalent to that of healthy children.[11] A more pronounced BA delay in children vs controls also has been reported in children with steroid-dependent nephrotic syndrome.[12]

ACTH	Adrenocorticotropic hormone	CD	Cushing disease
AICS	Adrenocorticotropic hormone–independent Cushing syndrome	CS	Cushing syndrome
		DHEA	Dehydroepiandrosterone
BA	Bone age	DHEAS	Dehydroepiandrosterone sulfate
BAZ	Bone age *z*-score	E2	Estradiol
BMI	Body mass index	IGF-1	Insulin-like growth factor-1
CA	Chronological age	UFC	Urinary free cortisol

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Smaller studies of BA specifically in pediatric patients with CS have yielded conflicting results, with some studies reporting delayed BA in the majority of patients and others reporting BA consistent with chronological age (CA) in the majority of patients.[13-15] Our study is the largest report of BA in children with CS to date. In addition, it is the first report comparing BA in children with CD vs AICS, and the first longitudinal BA analysis in children with CS. Based on the fact that in CD, children have elevated ACTH and elevated levels of adrenal steroids that in turn are aromatized to estrogens, we hypothesized that children with CD might have more advanced BA *z*-scores (BAZs) compared with those with AICS. We also hypothesized that body mass index (BMI), androgen excess, insulin resistance, and insulin-like growth factor-1 (IGF-1) excess may correlate with BA. As a secondary objective, we aimed to see how BAZ changed after surgical cure of CS.

Methods

Children with a diagnosis of CD (n = 93) or AICS (n = 31) were included in the study. Inclusion criteria included preoperative BA assessment and completion of diagnostic transsphenoidal surgery or adrenalectomy at the National Institutes of Health between January 1994 and January 2012. Of these 124 individuals, 64 had a follow-up BA film study performed at the 1-year follow-up, 5 of which demonstrated recurrence of CD, and those 5 children were excluded from the follow-up BA analysis. All studies were conducted under clinical protocol 97-CH0076, approved by the *Eunice Kennedy Shriver* National Institute of Child Health and Human Development's Institutional Review Board. Informed consent from parents (and assent from older children) was obtained for all children. Diagnosis of CD was confirmed as described previously.[16] A single radiologist blinded to the diagnosis read the BA data using the Greulich and Pyle atlas.[17] Advanced BA was defined as a BAZ of ≥ 2; delayed BA, as a BAZ ≤ -2. Presurgical BA and hormonal measurements were obtained at a mean of 1.2 ± 2 months before the date of surgery. Testicular volume was measured using a Prader orchidometer.

Hormone Assays

Androstenedione, dehydroepiandrosterone (DHEA), and DHEA sulfate (DHEAS) were measured by high-performance liquid chromatography/tandem mass spectrometry from 2006 to January 2013 and by radioimmunoassay before 2005 at Mayo Medical Laboratories, Rochester, Minnesota. Testosterone and IGF-1 were measured by chemiluminescence immunoassay with a Siemens Immulite 2500 analyzer (Siemens Medical Solutions Diagnostics, Los Angeles, California). IGF-1 *z*-scores were calculated using age-specific normal ranges provided by the National Institutes of Health Clinical Center's laboratory. Estradiol (E2) and fasting insulin were measured by electrochemiluminescence immunoassay on a Roche Cobas e601 analyzer (Roche, Basel, Switzerland). ACTH was measured by the Nichols Advantage immunochemiluminometric assay (Quest Diagnostics, Madison, New Jersey) before 2005 and with the Siemens Immulite 2500 analyzer thereafter. Diurnal plasma cortisol was measured by placing an intravenous catheter at least 2 hours before the test; cortisol levels were drawn at 2330 and 2400, and the patient was asleep to determine midnight cortisol values. Plasma cortisol was measured by chemiluminescence immunoassay. Twenty-four-hour urinary free cortisol (UFC) was averaged from 2 separate preoperative measurements by high-performance liquid chromatography/tandem mass spectrometry. To account for known sex- and age-based differences in androgen levels, values of DHEAS, DHEA, androsteonedione, E2, and testosterone were normalized and expressed as ratio of the patient's value to the mean value for age and sex.[18,19]

Statistical Analyses

Simple descriptive statistics and frequency distributions describe the data, which are reported as mean $\pm$ SD, median (IQR), or frequency (count). Comparisons of continuous data between groups (CD vs AICS, males vs females, advanced BA vs delayed/normal BA) were done using the *t* test or Wilcoxon rank-sum test, as appropriate. Where necessary, certain data were log-transformed for comparisons. The Fisher exact test was used to compare categorical data between groups. A 1-sample binomial test was used to compare the prevalence of advanced BA in children with CS with the expected prevalence of 2.5% in the general population. ANCOVA considered the role of sex in the comparison of certain clinical features, such as testosterone and E2 levels. Logistic regression modeling and correlation analyses were performed to assess the relationships between various clinical features and BA. Initial and 1-year follow-up data were compared using the paired *t* test and McNemar test. A *P* value $\leq .05$ was considered statistically significant. Data were analyzed using SAS version 9.2 (SAS Institute, Cary, North Carolina).

Results

Data from 93 children with CD (43 females; mean age, 12.3 ± 2.9 years) and 31 children with AICS (22 females; mean age, 10.3 ± 4.5 years) were analyzed retrospectively. The mean time of the presurgical BA and laboratory evaluation was 1.2 ± 2 months before the date of surgery. These and other demographic data are presented in **Table I**. Because AICS is known to be more common in females, it follows that our population had statistically significantly more females in the AICS group compared with the CD group. Comparing the 2 groups (**Table I**), the children with CD were older at the time of surgery and had a longer duration of symptoms. As expected, the children with CD had elevated ACTH levels, as well as higher DHEA and DHEAS levels. Similar results were found when these androgens were normalized and expressed as a ratio of the patient's value over the mean value for normal individuals of the same age and sex. Testosterone levels were not significantly different between the children with CD and those with