

Possible andrologic markers in elevated neonatal 17-hydroxyprogesterone

In this prospective study, we analyzed 30 male infants with increased neonatal 17-hydroxyprogesterone (17OH-P) (patients) and for comparison 52 age-matched healthy babies (control subjects) with the aim of investigating the hypothalamic-pituitary-testis axis in the first 6 months of life. Although T, FSH, and LH levels were not significantly different in patients and control subjects, inhibin B was higher in patients than in control subjects. Therefore we suggest a clinical follow-up of these babies during childhood and puberty to verify the evolution of their condition. (*Fertil Steril*® 2010;94:2350–2. ©2010 by American Society for Reproductive Medicine.)

Key Words: Inhibin B, 17OH-P, newborn, weight, male

During the first months of life, the hypothalamic-pituitary-gonadal axis is transiently activated in males, with increased gonadotropins, testosterone, and inhibin B levels (1, 2). Peak levels are observed at around the third month of life. Later on, serum hormone levels significantly decrease, remaining low until puberty (3–5).

Undetectable levels of inhibin B were observed in infants with bilateral absent testis, and lower levels were seen in infants with cryptorchidism, monorchidism, and testicular damage (4, 6, 7). Recently, low levels of inhibin B were also described in prepubertal boys with congenital adrenal hyperplasia (CAH), even in the absence of testicular adrenal rest (8). Inhibin B levels in males presenting neonatal increase of 17-hydroxyprogesterone (17OH-P) (~40% of all positives at neonatal screening for 21-hydroxylase deficiency [21-OHD] [9]) are presently unknown. This information, however, is interesting in view of recent studies reporting a high rate of infertility in patients with congenital adrenal disease (10–13). As a matter of fact, it is not known whether an

asymptomatic and untreated neonatal hyper-17OHP-emia can modify the inhibin B secretion, nor its consequences in adulthood.

Therefore, the aim of the present prospective study was to investigate the hypothalamic-pituitary-gonadal axis in the first 6 months of life in male babies with increased neonatal 17OH-P values, and to compare their data with those obtained in normal infants of the same age.

Thirty newborns with neonatal hyper-17OHP-emia (patients) were analyzed. Inclusion criteria were: male gender, gestational age (GA) >36 weeks, uncomplicated gestation and delivery, birth weight (BW) >2,500 g, absence of congenital disease, and increased 17OH-P levels at neonatal screening for 21-OHD. Exclusion criteria were: preterm delivery, classic salt wasting form of 21-OHD, and acute illness in the first days of life.

For comparison, we enrolled 52 age-matched healthy male babies (control subjects), with the same inclusion and exclusion criteria, except for displaying among the inclusion criteria values of 17OH-P within the normal reference range.

The study was conducted in compliance with the terms of the Helsinki II Declaration, and was approved by the Institutional Review Board of Verona. Informed written consents were obtained from the parents of the patients and control subjects.

The newborn screening 17OH-P cut-off level for term babies was set at 40 nmol/L. Positive babies at screening were recalled to confirm the result. For each patient, we recorded GA, BW, birth length (BL), and state of health. These infants were assessed every 3 months from birth to 6 months of life and submitted to complete physical examination. All of them underwent blood withdrawal to measure serum 17OH-P, electrolytes, FSH, LH, T, and inhibin B levels. Testicular volume was assessed using a standard Prader orchidometer. Testicular ultrasound scanning was performed at the first and third evaluations by the same radiologist.

For comparison, we assessed T, gonadotropins, and inhibin B in control subjects at approximately the same age as the patients.

Newborn screening for 21-OHD is based on 17OH-P fluoroimmunoassay on blood spots (AutoDelfia Neonatal 17OH-P Kit; Wallac Oy, Turku, Finland). Serum FSH, LH, and T measurements were determined by immunometric assay (Immulite 2000;

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Siemens, Erlangen, Germany). Serum 17OH-P was assayed by RIA (DSL-5000 Active 17 α -OH Progesterone Coated-Tube Radioimmunoassay; Diasorin, Saluggia, Italy), and serum inhibin B by ELISA (Active Inhibin B ELISA, Diagnostic Systems Laboratories, Webster, TX).

Statistical analyses were performed using SPSS 16.0 for Windows (SPSS, Chicago, IL). Comparisons between groups were performed by using Student *t* test or Mann-Whitney *U* test, and correlations by using Spearman or Pearson test, whenever appropriate.

No differences in patients' and control subjects' neonatal features were detected; however, during the clinical follow-up, patients showed a significant increased growth (Table 1). All patients' testicular volumes appeared normal for age, and they remained in the normal range for age during the entire follow-up. No differences were reported in their testis size compared with control subjects. Testicular ultrasound showed, in all of the subjects, both testes in the scrotum. None presented adrenal rest or alterations in testicular parenchyma. Hydrocele was found in 10 babies at first examination, bilateral in five of them and persistent in one.

All patients showed normal electrolyte values but increased 17OH-P serum levels at the first measurement (median 17OH-P: 37.5 [range 12.6–210.0] nmol/L). Then, 17OH-P levels decreased with age (median 10.1 [5.4–30.0] nmol/L and 2.7 [1.4–36.0] nmol/L at 3 and 6 months, respectively), reaching the reference range at the sixth month of life (14, 15). None of them needed treatment for hyper-17OHP-emia.

Whereas inhibin B was higher in patients than in control subjects, no significant differences in testosterone and gonadotropins levels were found (Table 1).

In the patients, we did not find any significant correlation between inhibin B, FSH, and T in the first period of life. Correlations were found between inhibin B and LH at the first serum evaluation ($r = 0.447$; $P < .05$) and between screening 17OH-P levels and inhibin B levels at the first month of life ($r = 0.617$; $P < .005$). The decrease of 17OH-P levels between the first and the third evaluations correlated with the increase in weight between the third and the sixth month ($r = -0.600$; $P < .05$). In the control subjects, no correlations were observed.

None of the patients presented conditions explaining their increased 17OH-P. No severe enzymatic deficiencies were evident at birth, and their androgen pattern allowed us to exclude a simple virilizing form of CAH, although we could not exclude a nonclassical form of the disease (9). Consequently, we hypothesized that the neonatal hyper-17OHP-emia was probably due to a slow enzymatic maturation in the adrenal steroidogenesis process.

In agreement with earlier studies (2, 5, 16), we did not find any correlation between FSH and inhibin B in the first months of life, whereas patients' LH and inhibin B, at the first serum evaluation, were positively correlated. These data seem to confirm the hypothesis of a role played by LH in the initial regulation of inhibin B synthesis and secretion (16, 17).

There is no evidence that increased 17OH-P levels in the first year of life modify fertility in later years, and data of inhibin B levels in newborns with congenital adrenal disease are lacking. Nevertheless, some studies described a higher risk of infertility

TABLE 1

Growth and hormonal data in patients and control subjects (mean \pm SD).

	Patients (n=30)	Control subjects (n=52)
Birth		
Weight (g)	3,251.5 \pm 362.0	3,141.4 \pm 460.3
Length (cm)	50.3 \pm 1.8	50.6 \pm 2.0
GA (wk)	38.7 \pm 1.3	39.1 \pm 1.2
Screening 17OH-P (nmol/L)	133.5 \pm 228.2 [†]	11.1 \pm 5.4
First month		
Weight (g)	3,857.2 \pm 574.1	3,886.8 \pm 550.6
Length (cm)	53.1 \pm 2.1	53.6 \pm 2.1
FSH (U/L)	1.4 \pm 1.0	1.4 \pm 1.6
LH (U/L)	3.0 \pm 2.1	3.3 \pm 2.4
T (nmol/L)	8.2 \pm 7.2	4.5 \pm 5.0
Inhibin B (pg/mL)	119.9 \pm 50.6*	89.5 \pm 37.4
Age (d)	25.7 \pm 9.2	25.2 \pm 11.7
Third month		
Weight (g)	6,063.4 \pm 673.7*	5,076.0 \pm 711.7
Length (cm)	60.6 \pm 1.7 [†]	56.3 \pm 3.0
FSH (U/L)	1.6 \pm 0.8	2.0 \pm 1.3
LH (U/L)	3.0 \pm 2.3	2.8 \pm 1.9
T (nmol/L)	5.8 \pm 3.1	2.7 \pm 2.9
Inhibin B (pg/mL)	183.1 \pm 87.8 [‡]	141.7 \pm 40.0
Age (d)	90.0 \pm 13.2	86.6 \pm 26.7
Sixth month		
Weight (g)	7,742.5 \pm 746.2*	6,981.9 \pm 1,268.7
Length (cm)	67.0 \pm 1.5	65.4 \pm 3.8
FSH (U/L)	0.8 \pm 0.5	1.0 \pm 1.3
LH (U/L)	0.8 \pm 0.6	1.2 \pm 1.3
T (nmol/L)	1.0 \pm 1.0	0.1 \pm 0.1
Inhibin B (pg/mL)	202.2 \pm 78.7* [§]	117.4 \pm 69.1
Age (d)	180.8 \pm 6.6	180.7 \pm 20.9

Note: 17OH-P = 17-hydroxyprogesterone; GA = gestational age.

* Patients vs. control subjects: $P < .05$.

[†] Patients vs. control subjects: $P < .001$.

[‡] Patients, first month vs. third month: $P < .005$.

[§] Patients, first month vs. sixth month: $P < .001$.

^{||} Control subjects, first month vs. third month: $P < .001$.

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in adult males with CAH, considering inhibin B to be an important index of fertility as a marker of Sertoli cell function (10–13, 18). Inhibin B was demonstrated to be a marker of Sertoli cells during childhood as well (2–4), and it was hypothesized that it might be a predictor of future fertility in male newborns (19). Recently, low inhibin B levels were described in prepubertal boys with CAH, suggesting a gonadal dysfunction in these patients, although the impact of this finding on future fertility remained unknown (8). In contrast, our most intriguing finding was the increased levels of inhibin B in infants with elevated neonatal 17OH-P values compared with control subjects.

High inhibin B levels were described in prepubertal patients with specific disorders in the androgens' biosynthesis or action. The cause of this finding is still unknown, but it was hypothesized that the deficit of testosterone action was the *primus movens*, followed by an increased daily production of FSH and/or its insufficient paracrine action on Sertoli cells (20, 21). Another speculative

interpretation of our data could involve Sertoli cells, which in case of androgens' excess might be stimulated by elevated neonatal 17OH-P levels, and consequently display a precocious maturation with active proliferation and increased production of inhibin B (22, 23). This hypothesis is indirectly confirmed by the correlation between screening 17OH-P values and inhibin B levels. However, the role of neonatal hyper-17OHP-emia in the possible precocious maturation of Sertoli cells and their relationship on future fertility remain unclear.

Infants with neonatal hyper-17OHP-emia had heavier weight and longer length than control subjects at 3 months of life, and this finding was confirmed at the sixth-month evaluation only for weight.

An earlier study described no differences in the growth during the first year of life in children affected by CAH undiagnosed at birth, suggesting the presence of relative resistance to androgen excess during this period (24). This finding was supported by the evidence of normal growth in other pathologic conditions with

elevated androgen levels (25). In contrast, some studies analyzed the effect in the uterus of androgen excess, reporting that BL of babies with CAH was higher than that of the normal population (26, 27). In the present patients, BW and BL were not significantly different compared with control subjects, but, over time, their weight and length exceeded those of the control subjects. Therefore, we can hypothesize that neonatal hyper-17OHP-emia influences the growth for some months after birth. The correlation between postnatal decrease of 17OH-P levels and the increase of weight between the third and the sixth months indirectly confirm this hypothesis.

In conclusion, we found that male infants with elevated neonatal 17OH-P values had higher inhibin B levels and increased weight compared with a control population until at least 6 months of life. Whether elevated inhibin B levels in the first months of life have an effect on testicular function later in life remains unknown. Further follow-up of these babies during childhood and puberty is useful to assess the evolution of their clinical condition.

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