Clinical Utility of Serum E2 Assayed by Means of a High-Sensitivity Kit in Children

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Abstract. To evaluate the usefulness of a high-sensitivity assay kit for E2, we measured the serum E2 levels in children. The value in a girl with true central precocious puberty was also evaluated before the LH-RH analogue treatment. The study subjects were 36 boys, ranging in age from 1 yr and 9 mo to 17 yr and 6 mo and 46 girls, ranging in age from 1 yr to 15 yr and 6 mo. The minimal detection limit was 1.4 pg/ml. The serum E2 level in the boys and that in girls were 10.8 ± 3.1 pg/ml (mean ± SEM) and 20.7 ± 10.7 pg/ml, respectively. The E2 level was correlated closely with bone age and chronological age. Even when the range of serum E2 was limited to an undetectable level (<10 pg/ml) by the conventional assay, the logarithmic value for the E2 concentration was correlated with bone age in boys (r=0.734, p<0.05) and in girls (r=0.404, p<0.05). The E2 level in girls with partial and true precocious puberty ranged from 2.6 to 8.7 pg/ml. In a patient, who was originally considered as premature thelarche and later turned out to be true central precocious puberty, the serum E2 level was 3.7 pg/ml before therapy, suggesting that high sensitivity to estrogen was the cause of the condition. With LH-RH analogue treatment, the E2 level declined to below the detection limit and the bone age did not change for 1 yr without loss of linear growth. These results suggest that the high-sensitivity kit is useful for monitoring the pathophysiology of E2 in children.

Key words: estradiol, high-sensitivity assay, children, radioimmunoassay, bone age, precocious puberty

Introduction

It is well known that E2 is the most potent estrogen. It plays a central role in pubertal development of girls, and also an important role in bone maturation and growth spurt even in pubertal boys (1). In a conventional radioimmunoassay (RIA) without preparatory extraction of steroid, the detection limit of the serum E2 concentration has ranged from 10 to 20 pg/ml. Such a detection limit is optimal for reproductive adult women, who have normal menstrual cycles or are potentially pregnant, but too high to monitor pathophysiological changes in serum levels of most prepubertal children.

Recently, highly sensitive RIA kits for measuring the serum E2 concentration have become commercially available, and they have made it possible to monitor the physiological changes in serum E2 levels throughout the
pediatric age. The present study was designed in order to evaluate the clinical usefulness of a high-sensitivity assay kit for E2 in a pediatric endocrine clinic. We measured the serum E2 levels in children with various pediatric endocrine disorders including simple obesity and precocious puberty. We examined the correlation between E2 and the chronological age or the bone age. In a patient, who was originally considered as premature thelarche and later turned out to be true central precocious puberty, the serum E2 level was at a prepubertal level in the putative diagnosis of true central precocious puberty, suggesting that high sensitivity to estrogen was the cause of the condition. This case is also presented here.

Subjects and Methods

The study subjects were 82 consecutive children, 36 boys and 46 girls. Their ages ranged from one year to 17 yr and 6 mo (mean age 9 yr and 2 mo). The boys (mean age, 10 yr and 7 mo) were older than the girls (mean age, 8 yr and 7 mo; p<0.02). They visited the Pediatric Endocrine Clinic of the University of Occupational and Environmental Health from May to September, 2000. Informed consent was obtained from each subject or parents as appropriate before taking blood samples for the E2 assay. The male subjects, whose ages ranged from 1 yr and 9 mo to 17 yr and 6 mo, consisted of 16 boys with non-endocrine short stature, 4 with GH deficient short stature, 8 with simple obesity, 2 with congenital adrenal hyperplasia and 6 with miscellaneous diseases. The female subjects, whose ages ranged from 1 yr to 15 yr and 6 mo, consisted of 9 girls with non-endocrine short stature, 5 with GH deficient short stature, 7 with simple obesity, 2 with congenital adrenal hyperplasia, 5 with Turner syndrome, 2 with true precocious puberty, 9 with partial precocious puberty and 7 with miscellaneous diseases.

The bone age was estimated from the radiography of the both hands for each study subject by the method of Greulich and Pyle. One well-trained investigator estimated all the radiography. Serum was separated by centrifugation and was kept frozen at −80 C until measurement. E2 was assayed by means of a commercial kit for high sensitivity RIA that was purchased from Diagnostic Products Corporation (Los Angeles, CA, U.S.A.). All samples were assayed in one assay. The minimal detection limit of the assay was 1.4 pg/ml. The E2 values of all but one sample were in the measurable range.

The data are expressed as means ± SEM. Since the data for serum E2 concentration were significantly skewed, they were transformed logarithmically before performing a statistic analysis. The difference between the means was estimated by the Mann-Whitney test. The values were considered to be statistically significant at p<0.05.

Results

The study subjects showed no sex-related difference in bone age (8.8 ± 0.8 vs. 7.8 ± 0.8 yr; mean ± SEM; boys vs. girls), height SD score (−1.25 ± 0.26 vs. −1.42 ± 0.26), or percentage overweight (13.9 ± 4.0 vs. 12.2 ± 3.7%). The serum E2 level in the boys was 10.8 ± 3.1 pg/ml and that in the girls was 20.7 ± 10.7 pg/ml. The difference between the sexes was not significant. The logarithmic value for the E2 concentration was correlated closely with chronological age in both boys (r=0.75, p<0.01) and girls (r=0.67, p<0.01) (data not shown). The logarithmic value for the E2 concentration was closely correlated with bone age in boys (r=0.85, p<0.001, Fig. 1) and in girls (r=0.66, p<0.01, Fig. 2). The E2 concentrations were lower than 10 pg/ml in boys whose bone ages were younger than 12 yr, and in girls whose bone ages were younger than 8 yr. Even when the range of the serum E2 was limited to the undetectable level (<10 pg/ml) by the conventional assay, the logarithmic value for the E2 concentration was correlated with bone age in boys (r=0.734, p<0.05)
and in girls (r=0.404, p<0.05).

The E2 in girls with partial precocious puberty ranged from 2.6 to 8.7 pg/ml. The serum E2 concentration in two patients with true precocious puberty was 2.7 pg/ml (under treatment) and 3.7 pg/ml (before treatment), respectively.

Case Report

A girl visited the clinic because of premature development of breast tissue at 11 mo old. Her bone age was equivalent to her chronological age and serum levels of LH and FSH were normal for age. The E2 level was undetectable by conventional assay at that time. She was diagnosed as premature thelarche. The bone age started to be advanced when she was 2 yr and 6 mo old (Fig. 3). Her bone age became 10 yr and 6 mo when she was 7 yr of age. The height SD score was +1.54 and the breast development was in Tanner stage IV, without signs of virilization or appearance of pubic hair. On LH-RH loading test, LH was <0.5 mU/ml at the baseline and 6.5 mU/ml at the peak value; FSH was 1.1 mU/ml at the baseline and 16.0 mU/ml at the peak value. The serum E2 level was 3.7 pg/ml. Nocturnal secretion during sleep was all <0.5 mU/ml for LH, and from 1.6 to 2.1 mU/ml for FSH. There was no abnormal silhouette in MRI of the hypothalamic pituitary region. The ovaries were slightly enlarged on ultrasonography. Vaginal smear showed a significant estrogen effect. Treatment with LH-RH analogue was started under a putative diagnosis of true central precocious puberty. The bone age did not change for 1 yr without loss of linear growth after that time, as shown in Fig. 3. The E2 level declined to below the detection limit, after the therapy was started.

Discussion

The serum E2 concentration assayed by a highly sensitive direct RIA increased gradually depending on the chronological and, more
particularly, the bone ages of the prepubertal children of both sexes. The correlation between the logarithmic value for E\textsubscript{2} and bone age was significant, even when the range of serum E\textsubscript{2} was limited to an undetectable level (<10 pg/ml) by the conventional assay. The serum E\textsubscript{2} concentration has been reported to be extremely high in cord blood, and then to gradually decline by 6 mo (2–4). It stays low before puberty but little is known about the prepubertal change in the serum E\textsubscript{2} level. Recently, a highly sensitive direct RIA method, as is used in the present study, has been developed (5), utilizing specific polyclonal antibodies. According to Ikekami \textit{et al.} (5, 6), the serum E\textsubscript{2} level was 1.24–5.58 pg/ml in boys under 3 yr of age, 0.68–3.97 pg/ml in boys aged 4 to 8 yr, 2.13–4.15 pg/ml in boys aged 9 to 12 yr, and 2.78–12.8 pg/ml in boys aged 13 to 16 yr. Those in females were 2.91–10.6 pg/ml under 3 yr of age, 2.58–4.16 pg/ml from 4 to 8 yr of age, 1.39–22.5 pg/ml from 9 to 12 yr of age and 6.13–92.0 pg/ml from 13 to 16 yr of age. In their study (5, 6), the serum E\textsubscript{2} level was low before puberty and always higher in females than in males in the same age groups.

Klein \textit{et al.} have developed an ultrasensitive recombinant cell bioassay for estradiol (7, 8). According to their assay system, the normal levels of E\textsubscript{2} (equivalent) in prepubertal boys and girls were 0.08±0.2 pg/ml (mean±SD) and 0.6±0.6 pg/ml, respectively, the value being much lower than those measured by RIAs. They noted that the serum E\textsubscript{2} (equivalent) level was higher in girls under 3 yr of age with premature thelarche (2.28±1.22 pg/ml) than in age-matched counterparts (0.9±0.95 pg/ml) (9).

A transient hypersecretion of FSH due to hyperfunction of the hypothalamic pituitary gonadal axis has been considered to be a mechanism for subtle E\textsubscript{2} hypersecretion leading to premature thelarche (10). The hormonal mechanism resulting in premature thelarche can be hypersensitivity of breast tissue to estrogen (11), transient autonomous E\textsubscript{2} secretion from the ovaries as seen in functional ovarian cysts (12), or secretion of precursor estrogen from the adrenal cortex (13). Among these hypotheses, the hypersensitivity of breast tissue to estrogen appears to be the most likely mechanism but the expression of estrogen receptor in the breast tissue of premature thelarche has not yet been studied because of difficulty in obtaining samples. It should be elucidated in further studies whether the hypersensitivity or hypersecretion is the mechanism for premature thelarche.

In the present study, the serum E\textsubscript{2} level in the patients with partial precocious puberty was similar to that in the other endocrine disorders. In the index case with true central precocious puberty,
the serum E₂ level before starting LH-RH analogue therapy was as low as the prepubertal level (i.e., 3.7 pg/ml), suggesting that hypersensitivity of not only the breast but also the bone tissues is the cause in this particular case. In this case, LH-RH analogue therapy suppressed the serum E₂ concentration to an undetectable level (i.e., <1.4 pg/ml), resulting in the cessation of advancement of bone age. Ikegami et al. (5, 6) also noted that the serum E₂ level was suppressed to <10 pg/ml during treatment with LH-RH analogue, and that it was rapidly restored to a higher level after cessation of the treatment. Klein et al. also observed with their ultrasensitive recombinant cell bioassay that LH-RH analogue administration suppressed the estradiol (equivalent) level dose-dependently in girls with central precocious puberty (9). Such a change in the serum E₂ level was too low for conventional RIA to monitor, indicating the clinical usefulness of the present assay system.

Acknowledgments

This work was supported in part by Grant-in-Aid #14570787 from the Ministry of Education, Culture, Sports, Science and Technology of Japan. Part of this work was presented in the 35th Annual Meeting of Japanese Society for Pediatric Endocrinology (Tokyo).

References