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Short Communication

Pitfalls in estradiol measurement by electrochemiluminescence immunoassay: A case study of a prepubertal girl with a falsely elevated serum estradiol level

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Highlights

- When elevated serum E2 levels, measured using immunoassays, appear inconsistent with the patient's clinical presentation or other endocrinological data, the measured serum E2 level may be falsely elevated.
- Based on the principles of ECLIA for E2, an insufficient reagent dose can cause falsely elevated serum E2 levels.
- If a falsely elevated serum E2 level is suspected, remeasurement, not only by LC-MS/MS but also by the same immunoassay, may be used to discriminate causes in the analysis, such as an insufficient reagent dose.

Key words: estradiol, falsely high value, electrochemiluminescence immunoassay, competitive assay, premature thelarche

Introduction

When elevated serum estradiol (E2) values, measured using immunoassays, appear inconsistent with the patient's clinical presentation or other endocrinological data, the measured serum E2 level may be falsely elevated. In general, the causes of the falsely elevated clinical values can be divided into two categories: samples and analyses. Serum E2 measurement is performed using an immunoassay, and various interfering substances present in the sample can affect E2 measurement. In our previous report (1), we described a case of a mature teratoma with a falsely elevated serum E2 level on electrochemiluminescence immunoassay (ECLIA). This was not recapitulated by liquid chromatography-tandem mass spectrometry (LC-MS/MS). We speculated a possible cross-reactivity between endogenous interfering substances in the sample and anti-E2 antibodies used in ECLIA, but the cause was unknown. We present the case of a three-year-old girl, with premature thelarche, who presented with a falsely elevated serum E2 level on ECLIA. The result

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that the falsely high value was not recapitulated by LC–MS/MS nor by the repeat ECLIA indicates a cause in the analysis, not in the sample. Based on the principle of the ECLIA for E2, we considered that insufficient reagent dose may cause falsely elevated serum E2 levels.

Case Report

A six-month-old female Japanese patient presented with thelache. Rapid growth acceleration was observed by the age of 2 yr (**Fig. 1**). At 2 yr and 11 mo, her bone age, determined via the Greulich-Pyle method (GP), was between four years and two months and five years (2). Using the Tanner-Whitehouse 2 method (TW2), her bone age was 4.8 yr (3). Her serum gonadotropin and E2 levels remained in the prepubertal (**Table 1**).

She was referred to our institution at the age of 3 yr and 8 mo with suspected precocious puberty. Physical examination revealed Tanner stage III breast, and Tanner stage I pubic hair development. Her height was 103 cm (+ 1.79 SD) and she weighed 15.9 kg (+ 0.93 SD). Her bone age was between 5 yr and 5 yr and 9 mo on GP, and 5.6 yr on TW2. No structural abnormalities were noted on magnetic resonance imaging of the head and pelvic ultrasound. On ECLIA (cobas e411 analyzer and ECLusys E2IV immune assay; Roche Diagnostics K.K., Tokyo, Japan), her serum E2 level was 128 pg/mL (reference interval for females aged 1–4 yr: < 5–21.9 pg/mL (4)), her serum LH level was < 0.1 mIU/mL, and her FSH level was 0.8 mIU/mL. A gonadotropin-releasing hormone stimulation test revealed a pre-pubertal response to gonadotropins (peak LH and FSH levels 9.2 and 24.0 mIU/mL at 60 min after the injection, respectively). On LC-MS/MS, the E2 level of the same serum sample was 3.6 pg/mL. The same sample was retested on the same ECLIA analyzer and reagents, yielding an E2 level of < 5 pg/mL (**Table 1**). Quality control data were within the normal range, and serum levels of other hormones measured using the same analyzer were normal. The analyzer did not display any error messages. No abnormal substances that could've affected the E2 measurement were detected after wiping the surface of the cobas e411 analyzer.

The patient was diagnosed with premature thelarche with accelerated bone age (5). On follow-up, no further exacerbations of accelerated height or bone maturation were detected (**Fig. 1**). Her serum E2 level, measured using ECLIA, remained < 5 pg/mL (**Table 1**).

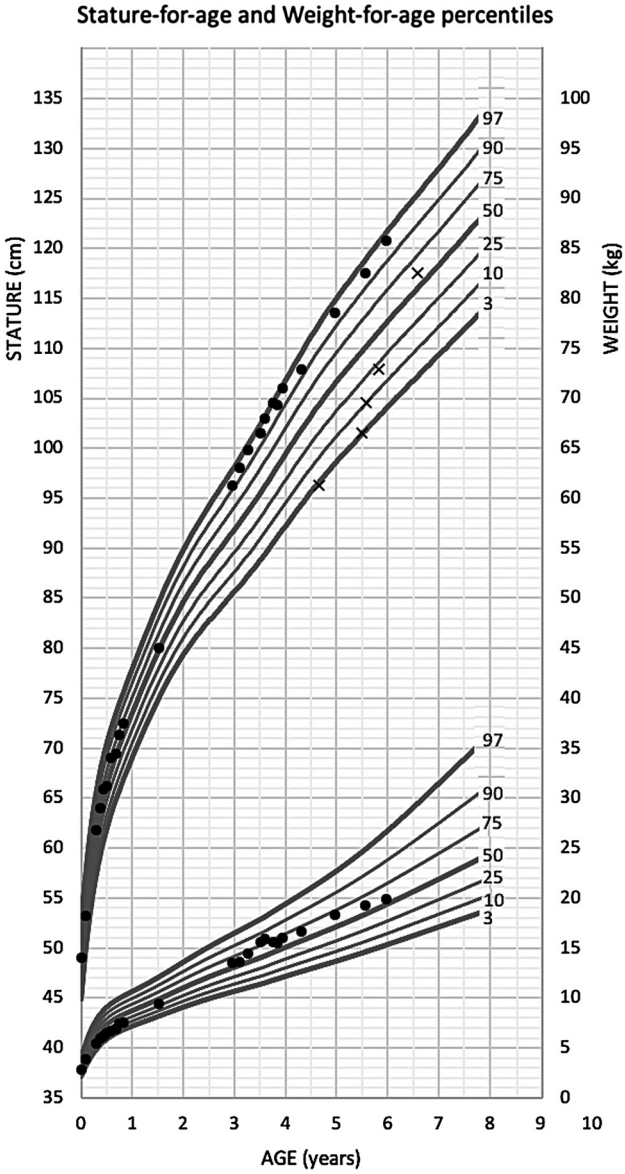

Fig. 1. The patient's growth chart. Circles indicate the patient's chronological age, while the x-mark indicates bone age.

Our patient showed high serum E2 level without suppression of serum gonadotropin levels after the GnRH stimulation test. Remeasuring the same serum sample using ECLIA or LC-MS/MS did not recapitulate

Table 1. Serum LH, FSH, and E2

Age (yr-mo)	2-11	3-8	3-9	4-3	4-11	
LH	0.1	< 0.1	< 0.1	0.1	0.1	IU/mL
FSH	1.4	0.8	2	2.3	1.9	IU/mL
E2 (ECLIA)	< 5.0	128	< 5.0	< 5.0	< 5.0	pg/mL
E2 (ECLIA reanalysis)	NA	< 5.0	NA	NA	NA	pg/mL
E2 (LC-MS/MS)	NA	3.6	3.5	NA	NA	pg/mL

ECLIA, electrochemiluminescence immunoassay; LC-MS/MS, liquid chromatography-tandem mass spectrometry; NA, not analyzed.

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the high E2 level. We speculate that the initial serum E2 level, measured using ECLIA, at 3 yr and 8 mo was falsely elevated.

Ethical statement

This study was conducted in accordance with the ethical standards of the institutional and/or national research committee, the 1964 Declaration of Helsinki, and its later amendments or comparable ethical standards. Institutional Review Board approval was not required for this case report. Written informed consent was obtained from the parents of the patient.

Discussion

The result that the falsely elevated E2 level was not recapitulated by LC-MS/MS nor by the same immunoassay indicates a cause in the analysis. Previous reports on falsely elevated serum E2 levels have discussed the presence of interfering substances in the serum samples. Although reanalysis using LC-MS/MS is important to verify the possible interfering substances, it cannot discriminate the possible causes in the analysis. Thus, reanalysis using the same immunoassay is necessary.

In light of the detection principle of ECLIA for E2, an insufficient reagent dose could lead to falsely elevated E2 values (**Fig. 2A**). E2 from the serum sample reacts with an anti-E2 antibody during the first reaction. The anti-E2 antibodies unbound to E2 from the sample reacts with the labeled E2. The complex of the anti-E2 antibody and labeled E2 detected by the emission signal intensity of the labeled E2. The intensity of the emission signal is inversely proportional to the amount of E2 present in the sample. Therefore, if the anti-E2 antibody (**Fig. 2B**) or labeled E2 (**Fig. 2C**) was insufficient for the assay, the intensity of the emission signal is reduced, leading to a falsely elevated E2 level (**Fig. 2D**). The insufficient reagent dose may have been caused by air bubbles. Conversely, based on this principle, if the serum sample dose was insufficient, the intensity of the emission signal would increase, and theoretically, the E2 measurement would be falsely decreased.

When elevated serum E2 levels measured by immunoassay appear inconsistent with a patient's clinical findings or other endocrinological data, remeasurement, not only by LC-MS/MS but also by the same immunoassay, can be used to discriminate causes in the analysis, such as insufficient reagent dose.

Conflict of interests: The authors have nothing to declare.

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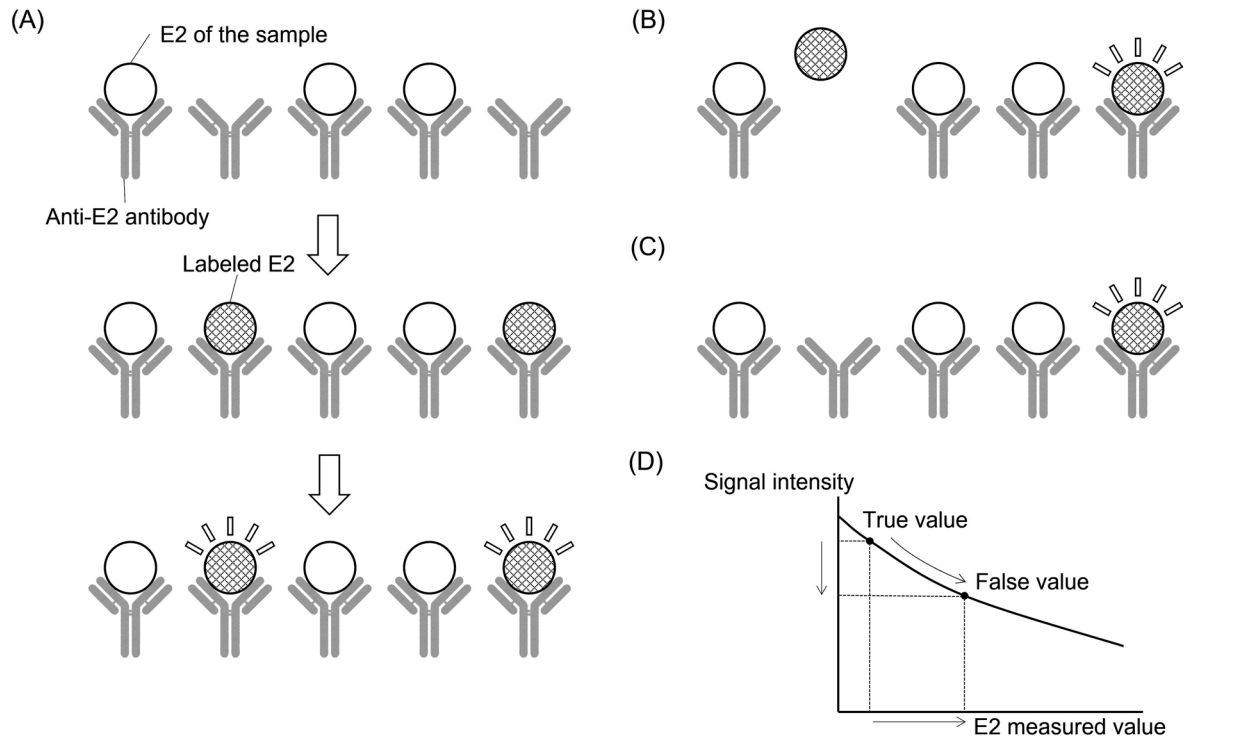

Fig. 2. Pitfalls on the electrochemiluminescence immunoassay (ECLIA) for estradiol (E2). (A) The assay's detection principle. (B) A pitfall in cases of insufficient dose of anti-E2 antibodies. (C) A pitfall in cases of insufficient dose of labeled E2. (D) Schema showing that emission signal intensity and the E2 measured value are inversely proportional.

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