Association of Biotin Ingestion with Interference of 25-Hydroxy Vitamin D, Parathyroid Hormone and Thyroid-Stimulating Hormone

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Method

The study included fifty children diagnosed as biotinidase deficiency (BTD) who were treated with oral biotin (5-20 mg/day).

Hormone Immunoassays: Serum 25-OHD, PTH and TSH measurements were made with two different assays in line with the manufacturers’ instructions and using original reagents: Beckman Coulter Access2 analyzer, Beckman Coulter ®, USA and Roche Cobas e411 Roche Diagnostics®, Meylan, France (biotin streptavidin-based method), before and after the biotin neutralization protocol.

HPLC and LC-MS/MS methods: The baseline plasma 25-OHD levels were measured using the HPLC approach involving a Thermo Scientific Dionex 3000 RS Liquid Chromatography, and a serum LC MS/MS analysis was performed using a Thermo Scientific TSQ access Max Triple Stage Quadrupole Mass Spectrometer (Thermo Scientific ®, San Jose, CA).

Serum biotin level measurement: Biotin levels were measured using a liquid chromatography-tandem mass spectrometry method (LCMS-MS, Thermo Scientific, San Jose, CA), before and after the neutralization procedure. The measurement range is 5.0–3000 µg/L for biotin.

Biotin neutralization method: We tested the adsorption of biotin to magnetic microparticles coated with streptavidin, which is a reagent that is supplied with the Cobas® assays kits provided by Roche, and that is available also from Sigma-Aldrich® (Saint Louis, MO, USA, ref: 11641778001).

Five volumes of the streptavidin reagent were centrifuged for 10 min (3000 g), after which the supernatant was carefully discarded, and one volume of the serum/plasma sample was added to the microparticle pellet, shaken for 1 hour at room temperature (100 rpm) and then centrifuged for 10 min (3000 g). The supernatant and assay were then collected with the necessary care.

Result

The median biotin concentration was 175.2 (94.0-307.1) µg/L and decreased to 0.9 (0.7-1.4) µg/L after neutralization with SMP.

No significant difference was detected in 25-OHD results between before and after SMP neutralization with Beckman Coulter, HPLC, and LC-MS/MS assays. The median 25-OHD level was decreased from 90.2 (35.9-105.3) ng/mL to 29.1 (22.6-37.6) ng/mL after SMP neutralization in Roche (p<0.0001) (Fig.1a).

Although there wasn’t any statistically difference between before and after SMP neutralization in PTH analysis with Beckman, the median PTH level was increased from 7.8 (1.6-21.6) pg/mL to 28.2 (22.5-41.9) pg/mL after SMP neutralization in Roche (p<0.0001). Cut-off value of serum biotin level in interference for Roche assays of PTH and 25-OHD levels were determined as 51.4 µg/L and 62.9 µg/L, respectively (Fig.1b).

A significant increase was detected in TSH level analyzed with Roche assays after neutralization (before neutralization 2.36 [1.85-3.00] mIU/L; after neutralization 2.74 [1.93-3.70] mIU/L; p<0.0001) (Fig.1c).

Conclusion

We confirm that PTH, 25-OHD and TSH results are affected by high biotin concentrations in Roche assays.

References


