Abstract title:
The worldwide epidemic of Vitamin D deficiency: are we contributing by using inaccurate and unreliable measurement methods?

Study question:
Is Vitamin D deficiency diagnosis biased by which measurement technique is utilized?

Summary answer:
25-hydroxyvitamin D (25OHD) serum concentrations are significantly lower when measured via Enzyme-Linked Immunosorbent Assay (ELISA) compared to Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS).

What is known already:
Vitamin D deficiency is widely reported in general population and in women undergoing ART, but lack of accuracy when measuring its metabolites remains as an unresolved issue. 25OHD is the most abundant vitamin D metabolite in the circulation, and is proposed to be the best indicator of vitamin D status. Although LC-MS/MS for serum 25OHD measurement is theoretically a more accurate and reliable technique than immunoassay-based methods, these are faster and less laborious, and are more commonly used for Vitamin D assessment in the general practice.

Study design, size, duration:
34 healthy women participating in our egg donation program were included during four months in this prospective, non-interventional cohort study. Serum samples were collected for quantification of 25OHD concentrations with the use of LC-MS/MS procedure and with ELISA. 25OHD levels according to IOM guidelines (<20, 20-30, and >30 ng/mL) were evaluated according each method results.
Participants/materials, setting, methods:
Serum was obtained in each subject, and then separated into two samples. 25OHD concentrations in one of the samples were measured via LC-MS/MS using a UPLC-TQ-S Xevo Waters system with a Waters Acquity BEH C18 (1,7μm 2,1 x100mm) column. A Vitamin D Enzyme-Linked Immunosorbent Assay (ELISA) kit (ab213966) Abcam for the quantitative determination of 25OHD was used for the other sample. A paired Wilcoxon test was performed for contrasting the mean differences between both techniques.

Main results and the role of chance:
All the cases were included and studied during autumn and winter months. None of them had taken vitamin D oral supplements during the last six months before sampling. Mean value for 25OHD concentrations in serum was 36.96±15.78 ng/ml when measuring via LC-MS/MS, and significantly lower when ELISA method was used (20.74 ± 21.73 ng/ml, p-value=1.255e-05). According to IOM guidelines, there was no Vitamin D deficiency in our studied population of 34 healthy women on reproductive age when LC-MS/MS is utilized, and most of them were in the sufficiency range of >30 ng/ml (n=23; 67.65%). In contrast, this population could be classified in the insufficiency range according to mean values obtained via ELISA method, most of them showing concentrations bellow <20ng/ml (n=23; 67.65%).

Limitations, reasons for caution:
The study population was a rather homogenous group of young, healthy women. These findings need to be confirmed in a larger, more diverse patient population.

Wider implications of the findings:
Utilizing ELISA to measure serum vitamin D levels results in an overestimation of vitamin D deficiency and may explain the increased prevalence in the population. Thus, LC-MS/MS should be considered as a more reliable procedure to measure Vitamin D in research and clinical practice.

Keywords:
Vitamin D
LC-MS/MS