Review Article

Technical Points in Vitamin D Measurement Assays

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Abstract

Background and aim: Major sources for vitamin D (Vit D3& Vit D2) in humans are skin and diet. Vitamin D is needed to maintain calcium concentrations within a narrow physiologic range and its status is assessed by measuring total serum levels of 25-hydroxyvitamin D, 25(OH) D metabolite. Despite the availability of many methods including High Performance Liquid Chromatography (HPLC), Liquid Chromatography -Mass Spectrometry (LC-MS/MS) and different immunoassays, the sensitivities and specifications of these vary considerably.

Methods: For the present paper, electronic databases including; PubMed, Scopus, Scientific Information Database, and etc during years 2000 to 2019 were selected and related papers were reviewed.

Results: The investigations indicated significant laboratory variation on the values generated for the 25(OH) D metabolite measurement in the same specimen using different methods and assays.

Conclusion: In summary, the choice to which assay or method to use for routine serum vitamin D determination will depend on the available equipment and expertise of each laboratory. In addition, to the contribution of different international programs for vitamin D metabolites measurement- to minimize inter assay and intra assay laboratory variations- it is suggested to use a suitable commercial serum control materials along with the manufacture's quality control material for vitamin D routine determinations. So that each laboratory can establish its own assay specific clinical decision limits based on the institute of medicine recommendations for low and upper safe limits of vitamin D.

Key Words: Vitamin D- Immunoassay- Accuracy- Precision- Sensitivity - Specificity.

Introduction

The vital function of vitamin D- mineralization of teeth and bones through regulation of calcium and phosphorus homeostasis- is essential for a large variety of cellular and metabolic processes in the body (1). Vitamin D can be obtained from dietary sources of vegetal (vitamin D2) or animal origin (D3). The molecular structure of vitamin D is closely allied to the classic steroid hormones (eg, estradiol, cortisol, and aldosterone) in that they have the same root cyclopentanoperhydrophenanthrene ring structure(2). Technically, vitamin D is a secosteroid because one of the rings of its cyclopentanoperhydrophenanthrene structure has a broken carbon-carbon bond. In vitamin D, this occurs in the 9, 10 carbon-carbon bond of ring B (2). Vitamin D3 as a vitamin or essential dietary component is found in dietary sources

such as fatty fish and dairy products. Furthermore, the major source of vitamin D3- contributing 90% of vitamin D intake- is obtained via a photosynthetic reaction in the dermis of the skin (3-4). In humans vitamin D3 can either be made in the skin from a cholesterol like precursor (7-dehydrocholesterol) by exposure to sunlight or artificial ultraviolet radiation. Thus, vitamin D3 can be endogenously produced. As long as the individuals has access to adequate sunlight on a regular basis, they might not need to obtain this vitamin from the diet. However, dermatologists are concerned that individuals with extensive UV radiation exposure could have an increased risk of skin cancer or melanoma (5). Vitamin D2 is available in limited amounts from plant sources (Ergosterol) and in some supplements. Regardless of the route of entry, 99% of endogenous or dietary vitamin D through vitamin D binding proteins, and to a lesser extent by albumin travels to the liver, and undergoes first hydroxylations that produces corresponding 25-hydroxyvitamin-D; 25(OH)D. The 25(OH) D is the most abundant circulating metabolite of the vitamin D. In addition, it is a very sensitive measure of vitamin D stores in the body obtained from both UV radiation and dietary intake over long periods (6). Vitamin D and 25(OH) D have no established bioactivity (3). In the kidneys second hydroxylations occur and 25-hydroxyvitamin-D-1α-hydroxylase converts 25(OH) D to the biologically active hormone, 1, 25-dihydroxyvitamin-D; 1, 25(OH) 2D (6). This form is a calcium regulating hormone which acts to maintain serum calcium through its effects on intestinal calcium absorption and through a complex series of inter relationships with serum phosphate and parathyroid hormone. Furthermore, it is believed to act on target cells similarly to the way a steroid hormone would act namely by interacting with vitamin D receptor (7-8).

Vitamin D deficiency was shown to be prevalent in several countries and its deficiency (9) is associated with osteoporosis and osteomalacia (3, 10-12). Furthermore, it is associated with decreased muscle strength (13), cancer (14), cardiovascular disease (15-16), rickets in children (17), and increased overall mortality rate (18).These have contributed to the need for determining vitamin D status in a reliable way.

Studies have indicated that despite the availability of many different methods for 25(OH)D metabolite determination including; high performance liquid chromatography (HPLC), radioimmunoassay (RIA), automated immunoassays, and liquid chromatography-tandem mass spectrometry (LC-MS/MS), there is significant laboratory variation among results obtained for the same specimens (19).Furthermore, problems with precision, accuracy, specificity, and sensitively in long term surveys were noted (20-21).Currently measurement of vitamin-D metabolites by LC-MS/MS detection (22-23) has been established as the gold standard methodology. This method offers better accuracy at medical decision levels to correctly classify patients as vitamin-D deficient and sufficient (3). However, factors such as availability, ease of use, cost, small sample volume, and rapid turnaround time of automated immunoassays, have caused majority of clinical laboratories to use these assays for 25(OH)D metabolites determination.

Methods

For the present paper, electronic databases including; PubMed, Scopus, Scientific Information Database, and etc during years 2000 to 2019 were selected and related papers on the topic were reviewed.

Results

Vitamin D is required to maintain blood levels of calcium and phosphate that in turn is needed for the normal mineralization of bones, muscle strength, and general cellular function in all cells of the body. Investigations though related papers indicated in the recent years the knowledge about vitamin D and methodologies for its metabolites measurement in the healthy population has significantly increased. This is due to an increased awareness of vitamin-D deficiency and its potential association with many diseases beyond its role in maintaining bone health (3, 16). Although LC-MS/MS is considered the gold standard for 25(OH)D testing, according to the Accuracy-Based Vitamin-D 2016 Survey, only 74 out of 364 US laboratories used LC-MS/MS for 25(OH)D determination (20-21,23). It has been also indicated that this might be due to the labor, complex nature of development, implementation of clinical LC-MS/MS assays, and slower turnaround time for their adoption (23). In regard to immunoassays, technical innovations in sample processing and analysis – considering speed, and exact incubation time – have contributed to some improvement in the accuracy, and precision of the obtained results. In addition, it has caused 90% of laboratories to perform their routine 25(OH) D analyses by automated assays (3). Although evaluation of these automated assays still has raised concerns about their precision, accuracy and specificity (22-23). Furthermore, many different international programs have been established and used by laboratories to monitor the performance of laboratories using various methods for determination of 25(OH) D (24-26). It has been also indicated that immunoassays manufactures use different standards and calibrators (22, 27-29). Their control materials in most immunoassay kits including Radioimmunoassay (RIA) and Enzyme Linked Immunosurbent Assay (ELISA) only indicates whether there has been a general problem with the assay performance or not and does not guarantee whether the result is accurate because the control value came within the specified range.

Conclusion

In summary, the choice to which assay or method to use for the routine serum vitamin D metabolites measurement will depend on the available equipment and expertise of each laboratory. In addition, to the contribution of the international programs for the vitamin D metabolite determination - to minimize inter assay and intra assay laboratory variations- it is suggested to use suitable commercial serum control materials along with the manufacture's quality control material. So that each laboratory can establish its own assay specific clinical decision limits based on the institute of medicine recommendations for low and upper safe limits of vitamin D (30).

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