25- Hydroxyvitamin D: Explosion in Clinical Interest and Laboratory Requests

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25-hydroxyvitamin D, 25(OH)D is the most abundant vitamin D metabolite in the circulation, representing the best indicator of the nutritional status of this fat-soluble vitamin. Two distinct forms exist: 25(OH)D3 from cutaneously derived vitamin D (cholecalciferol), the predominant natural source of vitamin D in humans and 25(OH)D2 from vitamin D2 (ergocalciferol), derived almost entirely from supplementation or fortification of food.[1] Worldwide, there has been an explosion of interest in the physiological, pathological, therapeutic and laboratory aspects of 25(OH)D. Request for its measurement has increased dramatically over the last few years with an annual increase of about 80-90%.[2, 3] At the Clinical Biochemistry Laboratory of the Royal Hospital, Muscat, Sultanate of Oman, the annual request rate for serum 25(OH)D during 2009 was at a much higher degree compared to 2007. The vast majority of patients tested were deficient in 25(OH)D.

There is growing awareness for the role of vitamin D; not only for its role in metabolic bone disease, but also, the increasing recognition for its association with a variety of diseases. Several randomized controlled trials have revealed that vitamin D deficiency has been linked to the development of different chronic diseases such as cardiovascular diseases, autoimmune diseases, diabetes mellitus, neuromuscular dysfunction, chronic kidney diseases, different cancers, infections, and gynecological problems.[4, 5] Data from these studies mentioned earlier have demonstrated that circulating vitamin D is an important reflector of the total mortality risk.[6] A recent prospective cohort study by Zittermann *et al.* in a specialized heart centre revealed that patients in the lowest quintiles of 1,25-dihydroxyvitamin D (1,25(OH)D, also termed calcitriol) and 25(OH)D were more likely to have coronary heart disease, heart failure, hypertension, diabetes mellitus or renal failure compared to patients with higher concentrations of 25(OH)D.

The study also showed that low serum concentrations of 1,25(OH)D and 25(OH)D were related to higher 1-year mortality risk, while there was a significant decrease in 1-year mortality risk in patients with higher serum concentrations of vitamin D. The results were also consistent in patients representing different risk factors and multivariate risk adjustments such as age, body mass, smoking, aspirin use, renal function, inflammatory markers, and various co-morbidities.[7] Other studies such as the study by Dobnig et al. which focused on the levels of vitamin D and cardiovascular mortality and a study by Wolf et al. which studied the levels of vitamin D and mortality in patients on hemodialysis also showed similar findings and have also confirmed such an association.[6,8] Furthermore, meta analysis of different randomized controlled trials have revealed that vitamin D supplementation has been linked to lower total mortality in subjects with low 25(OH)D concentrations compared with un-supplemented individuals.[9] Thus, it is worth providing 25(OH)D therapy or supplementation to high risk individuals without necessarily measuring their serum 25(OH)D concentrations, which may not be available at many laboratories.

In addition to the increasing awareness regarding the key role of 25(OH)D in the maintenance of many physiological processes and recognition of its deficiency as a growing health problem, an analytical verification has to be addressed. Although there is no consensus on the optimal levels of serum 25(OH)D, most experts recommend that the standard level which confers its optimum physiological protective role and provides the full advantages of vitamin D health benefits is ≥75nmol/L (30 ng/ml).[10] Vitamin D status has been defined at different 25(OH)D cut-offs, with levels: 50-74nmol/L (20-30 ng/ml) as suboptimal, 25-49 nmol/L (10-20 ng/ml) as insufficiency, <25 nmol/L (10 ng/ml) as deficiency and <12.5 nmol/L(5 ng/ml) as frank deficiency. Levels in the range of 75-250nmol/L (30-100ng/ml) reflect adequacy/sufficiency, however vitamin D intoxication is rare and may be observed when 25(OH)D level is >375nmol/L (150 ng/ml) or even at higher levels.[5, 10] With the use of such definitions, it has been estimated that one billion people worldwide have vitamin D deficiency or insufficiency, 40-100% of US and European elderly men and women are deficient in vitamin D and more than 50% of postmenopausal women taking medication for osteoporosis had suboptimal 25(OH)D levels <75 nmol/L (30 ng/ml).[5] These figures further re-inforce the importance of supplementing high-risk individuals with 25(OH)D therapy irrespective of their serum 25(OH)D levels.

Several techniques are used to measure serum 25(OH)D levels, these include; liquid chromatography/tandem mass spectrometry (LC-MS/MS), gas chromatography-mass spectrometry (GC-

MS), high performance liquid chromatography (HPLC), radioimmunoassay (RIA), enzyme-linked immunosobent assay (ELISA) and chemiluminescence immunoassay particularly electrochemiluminescence immunoassay (ECLIA).[11] There are concerns regarding the accuracy, lack of correlation between the different assays, and limitations of certain assays, particularly immunoassays to measure all forms of vitamin D.[11,12,13] Hence, it has been recommended to use common standard material with different vitamin D2/D3 concentrations provided by a commercial manufacturer.[14,15] This has been adopted by the UK-based Vitamin D External Quality Assessment Scheme (DEQAS), the largest vitamin D proficiency testing program established in 1989 with currently more than 600 registered participants (www.deqas.org). The scheme is aimed to monitor the performance of 25(OH) D assays and to provide a unique opportunity for the assessment of analytical performance, accuracy and specificity of 25(OH)D methods of the users.[16]

Although there are concerns relating to the accuracy of all methods besides their poor precision, LC-MS/MS appears to have relatively better accuracy and may be considered as the reference method.[13,17] Moreover, the credibility of chromatographic measurement of vitamin D may suffer a further problem related to vitamin D standard preparation in different matrices, however, these methods appear to be the most superior.[15,16] Nevertheless, the equipment is costly and requires a level of training and expertise that may be beyond the scope of many laboratories. Other methods such as RIA, ELISA and ECLIA are less technically demanding, more readily available, and can be automated with high throughput and reproducible results. Automated platforms are available including DiaSorin Liaison Platform (using ELISA) and Roche Modular E170 Analyzer (using ECLIA). These technologies appear to be attractive and have become increasingly available with the majority of epidemiological surveys based on such methods particularly RIA. However, some reports have undermined the non-chromatographic methods for 25(OH)D2. This is particularly critical as vitamin D supplements that contain only vitamin D2 may not be detected and therefore, follow-up of vitamin D deficient patients who are on D2 replacement or prophylactic therapy will be challenging, leading to inappropriate supplementation, inaccurate monitoring or possible misdiagnosis.[18,19,20] Despite being reported by many workers, this problem may be underestimated by the manufacturers or physicians alike.[21,22] Vitamin D2 is less physiologically active than vitamin D3, and may be less commonly available in supplementations.[23] Furthermore,, many physicians are unaware of the type of 25-hydroxyvitamin D prescribed to patients and many vitamin D preparations provided in pharmacies do not contain such details. Therefore, measurement of both forms is important in validating 25(OH)D assays, and it is recommended to use a 25(OH)D assay that measures both 25(OH)D2 and 25(OH)D3, and the sum concentration is reported.[24]

In conclusion, there has been an rapid increase in interest for the role of 25(OH)D in health and disease, with growing awareness for its deficiency in the development of different chronic diseases and its independent association with all-cause mortality. The protective effect of vitamin D supplementation in high-risk individuals makes it worth providing 25(OH) D therapy or supplement to high risk individuals, even without necessarily measuring serum 25(OH)D which can be offered to selected patients. Clinicians must be aware of the formulation of vitamin D they are prescribing when monitoring patients who are at risk. Different analytical methods are now increasingly becoming available in the laboratories for serum 25(OH)D measurement, however, they may not be able to meet the demand for vitamin D test requests. Overall, the disadvantages of the analytical techniques needed for standardization, quality assurance and the lack of specificity in differentiating measurement of vitamin D2 and D3 is an important consideration for both manufacturers and consumers.

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