Editorial

Defining vitamin D deficiency, using surrogate markers

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In endocrine praxis, all deficiency is defined by a combination of trophic hormone and target hormone. Why then, is vitamin D deficiency (VDD) defined by an isolated value of storage hormone serum 25-hydroxu vitamin D (25OHD), rather than its active hormone, or its relation with target action of vitamin D? Are better surrogate markers available for the definition of VDD? This editorial seeks an answer to this seemingly simple answer.

VDD has been defined as serum 25OHD levels <20 ng/ml by Institute of Medicine Guidelines.[1] In addition, Endocrine Society Practice Guideline defines vitamin D insufficiency (VDI) when serum 25OHD levels are between 20 -<30 ng/ml.[2] The main physiological function of vitamin D is maintenance of calcium homeostasis. This is carried out mainly by its effect on calcium absorption, which is evident by reversal of all effects of vitamin D-resistant rickets type-II by intravenous calcium.[3] In addition, vitamin D has been associated with maintenance of bone health in association with parathyroid gland.[4] Although the extra-skeletal effects of vitamin D have been highlighted in many observational studies, their relevance has not been established conclusively.[5] Hence, the target organ for action of vitamin D is the intestine, and its target function, calcium absorption.

Surrogate Marker 1: Calcium Absorption

Can VDD be defined by its target function, *viz*., calcium absorption? Calcium absorption can be determined by calcium balance studies or by isotope technique using stable or radioactive tracers and either single- or dual-tracer.[6] Calcium balance studies required an equilibration period on a standard calcium intake after fecal collections that were pooled and analyzed on a 3-6 d basis. These studies are expensive, time-consuming, and ill-suited for routine use. Single oral tracer methods require fecal collections or the use of mathematic calculation called deconvolution analysis.[7] However, this methodology is less accurate and not useful in children. In the dual-tracer method, one isotope is given orally and another administered intravenously. This method is rapid, accurate, and does not require collection of stool. Dual tracer method correlates highly with calcium absorption,[5] and is the gold standard for measuring calcium absorption. This method measures fractional calcium absorption (FCA) of ingested calcium.

Measurement of FCA has not been used to define VDD because of several limitations. Firstly, these methods are not readily available, require expertise and availability of radiocalcium tracers, hence, cannot be used in routine clinical practice. Secondly, FCA varies widely with calcium intake, as highlighted by Garg *et al*.[8] Lastly, the relation between serum 25OHD and FCA is complex and still not completely understood.[6] Although some reports indicate optimization at specific concentrations of serum 25OHD above those commonly found in the population, others differ.[9-11] It is widely believed that extremely low serum 25OHD values (<8 ng/ml) are associated, in both children and adults, with decreased calcium absorption.[10,11] It is clear, therefore, that as there is no clearly delineated negative feedback pathway, measurement of calcium absorption cannot be used clinically to define VDD.

Surrogate Marker 2: Parathormone

As explained above, the most important effect of

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vitamin D is calcium absorption. In VDD, with decreased calcium absorption, systemic adaptation comes into play to maintain calcium homeostasis. The first adaptation, as per current theory, is an increase in parathormone (PTH), which increases serum calcium acutely by mobilization of calcium from bones. This makes PTH an indirect, but early, indicator of VDD, though in all probability, it is not directly involved in calcium absorption.[8]

In the endocrine clinic, one must be aware of limitations in measurement of PTH. PTH is a polypeptide, which is degraded quickly by peptidases present in blood, with half-life of only 3-5 minutes.[12] Due to this, collection of blood requires a cold syringe, which should be immediately cold centrifuged and stored at −20°C till measurement is performed.[13] Nevertheless, with proper precautions taken, PTH can be used as an indicator of VDD. Another limitation is the cut-off point for PTH to be used to define secondary hyperparathyroidism (SHPT) associated with VDD. The upper-off limit of commercially used PTH assay is ~65 pg/ml, which is taken as a cut-off for SHPT. The upper limits of PTH provided by manufacturers are probably too high because VDD is often not accounted for. In fact, the reference range of a second generation PTH assay decreased from 65 pg/ml to 46 pg/ml when subjects with VDD were excluded in one study.[14] Similarly, a third generation PTH assay which is currently used resulted in 20% lower PTH value with reference interval of 7-36 pg/ml in normocalcemic individuals.[15] Recently, a laboratory-based analysis of more than 300 000 serum 25OHD and PTH pairs revealed an age-related increase in PTH with similar serum 25OHD levels.[16] Hence, it becomes imperative to define age-related cut-off for PTH for the population, which is vitamin D sufficient. Lastly, one must also consider missing normocalcemic primary hyperparathyroidism,[17] particularly in a VDD-endemic population. Such subjects may be mislabeled as SHPT if calcium levels are within normal range. In a recent study conducted on relation between serum 25OHD, PTH and bone mineral density in Indian adolescents and adults (>50 years), the cut-off of PTH defined by significant deterioration of BMD was 35 pg/ml for adolescents and 55 pg/ml for adults.[18]

Surrogate Marker 3: Bone Mineral Density

Vitamin D is extensively linked with bone health. Can bone mineral density (BMD) or bone turnover markers be used to define VDD? A recent review analyzed the association of specific circulating 25(OH) D concentrations with bone health outcomes in children, women of reproductive age, postmenopausal women, and elderly men.[4] A positive association between serum 25OHD and BMD was found in adolescents, but not in postmenopausal women and elderly subjects. There is evidence of a dual, dose-dependent, antipodal effect of vitamin D on bone health. Experimental studies on bone cell culture media suggest that activated VDR stimulate osteoclastogenesis at lower, but osteoblastogenesis at higher concentration.[19] As the primary role of vitamin D is calcium homeostasis, VDD and associated low calcium absorption will stimulate active vitamin D metabolites to increase bone resorption and maintain circulating calcium levels.[20] This will also be reinforced by raised PTH levels in presence of VDD. In a scenario of vitamin D sufficiency and adequate calcium supply, active vitamin D metabolites will facilitate calcium deposition in bone.

Hence, a low BMD can be used as a marker of VDD. As highlighted by Garg *et al.*, a Z-score of -1 in adolescent and T-score of −1.5 in elderly is associated with VDD and SHPT.[8] However, there are many vitamin D-independent variables which affect BMD. Moreover, VDD will take a long time to affect BMD, and a short duration of VDD may not adequately reflect end organ damage. Hence, low BMD when present can be taken as indicator of VDD, but absence of it will not rule out presence of VDD.

Surrogate Marker 4: Bone Turnover Markers

There are several bone turnover markers, which are increased in patients with rickets/osteomalacia i.e. serum alkaline phosphatase (total or bone specific), osteocalcin, and serum N-terminal propeptide of type 1 procollagen (P1NP), beta C-terminal cross-linked telopeptides of type I collagen (b-CTX).[21] An inverse correlation of serum 25OHD and these bone turnover markers[22,23] have been observed in most studies; yet other authors have also reported no association.[24-26] There has been low accuracy of bone turnover markers in defining VDD in receiver operator curve analysis[27] and low sensitivity in detecting VDD.[28] No definite cut-off levels of serum 25OHD are defined, at which these bone turnover markers start rising. Bone turnover markers are also affected by non-vitamin D factors such as age, sex, puberty, and other systemic and bone disease.

Moreover, ethnic specific normative data will be required for these markers for vitamin D sufficient population. These data suggest that increased levels of bone turnover markers are a good indicator of VDD, but normal levels do not exclude presence of VDD.

Conclusion

Current evidence clarifies that serum PTH levels are, at present, the best indirect indicator of systemic effects of VDD. Till a better and simpler indicator of VDD is available, serum 25OHD levels should be interpreted with PTH levels, rather than in isolation. This will also decrease the prevalence of VDD by 50% globally as only 50% of subjects with serum 25OHD levels <10 ng/ml showed presence of SHPT.[16-29] There is urgent requirement of studies specifically comparing the subjects with serum 25OHD with and without SHPT. There is also need to study the beneficial effect of vitamin D supplementation on clinical, biochemical, and bone health parameters in these subjects, rather than blindly aiming to raise vitamin D levels to arbitrarily set levels.

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