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Review

Standardized measurement of circulating vitamin D [25(OH)D] and its putative role as a serum biomarker in Alzheimer's disease and Parkinson's disease

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ABSTRACT

The current review provides an overview on the development of 25(OH)D measurement standardization tools over the last three decades and clarifies whether there is a role as a serum biomarker for vitamin D in neurological diseases. In the past, a lack of internationally recognized 25(OH)D reference measurement procedures and reference standard materials led to unstandardized serum total 25(OH)D results among research and clinical care laboratories. The vitamin D Standardization Program (VDSP) has been introduced in 2010 to address this problem, however, vitamin D External Quality Assessment Scheme (DEQAS) reports still show substantial sample- to- sample variability. Further, immunoassays, which are mainly used in clinical care laboratories, display analytical issues, including matrix-effects interferences, which cannot be overcome by the standardization process. Hence, liquid chromatography-tandem mass spectrometry (LC/MS-MS) methods should be used to measure 25(OH)D. Low vitamin D serum levels have been found in patients affected by Alzheimer's disease and Parkinson's disease, suggesting a role for vitamin D as a serum biomarker in these diseases. However, few studies reported 25(OH)D standardized results, thus, no clear evidence on the potential role of 25(OH)D serum levels in these diseases exists.

1. Introduction

Serum total 25-hydroxyvitamin D [25(OH)D], the sum of 25-hydroxyvitamin D2 [25(OH)D2] and 25-hydroxyvitamin D3 [25(OH)D3], is the best biomarker to assess vitamin D status [1]. Vitamin D is a secosteroid mainly produced in the skin by the action of sunlight on 7-dehydrocholesterol. The active hormonal form, 1,25-dihydroxyvitamin D [1,25(OH)2D], exerting biological activity, is obtained after two hydroxylation steps (Fig. 1) [2]. Upon binding its nuclear receptor, vitamin D Receptor (VDR), active vitamin D regulates the expression of hundreds of genes [3], regulating several biological processes [4,5]. All key enzymes involved in the metabolism of vitamin D are Cytochrome P450 enzymes, although these are mainly known to catalyse oxidation/reduction reactions and liver drug catabolism [6].

Classically, vitamin D has a well-known role in skeletal health and calcium/phosphorus homeostasis. Beyond bone metabolism, a large amount of data proves that active vitamin D plays a part in the modulation of the immune response [4]. Also, many studies documented that 1,25(OH)2D is involved in the regulation of brain development and function in adulthood [5], through the interaction with the surface receptor protein disulphide isomers family A members 3 (PDIA3) (Fig. 2) [7,8].

Low 25(OH)D circulating levels have been studied in patients affected by different diseases. Serum 25(OH)D has been proposed as a biomarker of disease severity for infections, with well-established biomarkers [9–13]; likewise, a role for vitamin D as a serum biomarker in autoimmune diseases has been documented [14–17]. Recently, an increasing body of data shows that vitamin D could be used as a serum biomarker in neurodegenerative disorders, along with inflammatory and cardiovascular markers [18–28]. The considerable efforts made by

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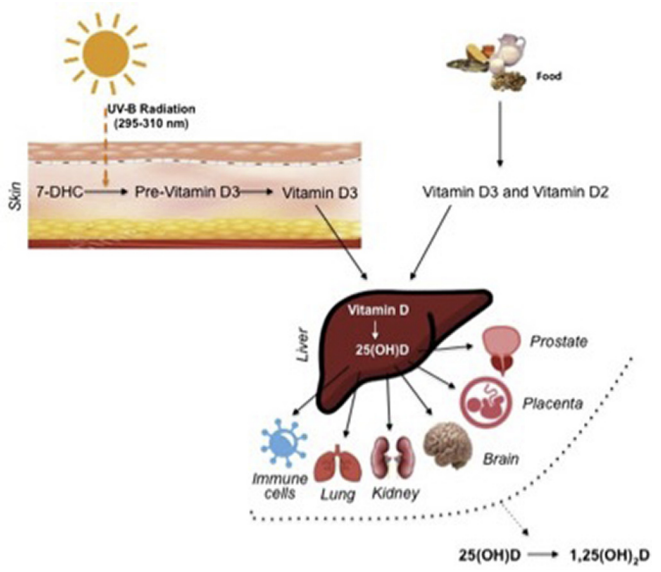

Fig. 1. Vitamin D synthesis.
Vitamin D includes two natural compounds: vitamin D2, deriving from the diet, and vitamin D3, which derives both from the diet and endogenous synthesis. Ultraviolet B rays (295–310 nM) transform the cutaneous precursor 7-dehydrocholesterol into vitamin D3 (cholecalciferol), which requires two sequential hydroxylations to form the active vitamin D3. The first hydroxylation produces 25(OH)D in the liver through the action of 25-hydroxylase [1,25(OH_2)D]. 1-α-hydroxylase carries out the second hydroxylation forming 1,25(OH_2)D in the kidney, prostate, placenta, lung, brain, and immune cells. UV-B: ultraviolet B rays; 7DHC: 7-dehydro-cholesterol.

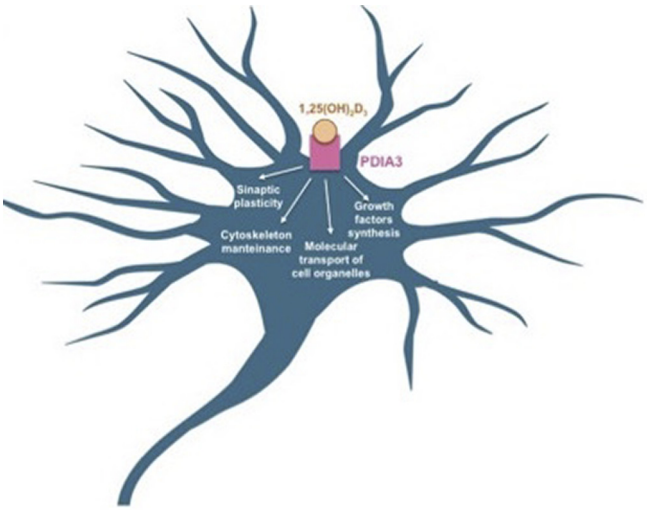

Fig. 2. Vitamin D influences brain development and function in adulthood.
vitamin D modulates neuronal cell processes by regulating the expression of many proteins.

researchers to address the issue of vitamin D role in neurological disease can be explained by the multifaceted role that the hormone plays at the brain level, as illustrated below.

2. The role of vitamin D in brain function: A summary

VDR, as well as 1-α-hydroxylase, is widely expressed in both embryonic and adult brain [29]. Neurons and microglia synthesise active vitamin D, which locally acts in a paracrine/autocrine fashion, regulating differentiation, proliferation and survival in these cells [29]. This action results in the modulation of many processes, including synaptic plasticity, neurotransmission and neuroprotection (Fig. 2). Notably, active vitamin D regulates the expression of many proteins involved in i) synaptic plasticity, counting drebrin, growth-associated protein 43 (GAP43) and connexin 43, ii) cytoskeleton maintenance, including neurofilament, tubulin, microtubule-associated protein-2 (MAP2), iii) molecular transport of cell organelles, with creatine kinase b, kinesin, RhoA, dynactin [30,31]. Further, vitamin D up-regulates the expression of nerve growth factor (NGF), neurotrophin 3 (NTF3) and glial-derived nerve growth factor (GDNF) [32,33] (Fig. 2A). Vitamin D also influences dopaminergic circuits operation and connectivity, and substantia nigra, a key area for the production of dopamine, strongly expresses 1-α-hydroxylase [34]. Besides, VDR is expressed in striatum, accumbens nucleus and prefrontal cortex [35], which are the brain areas forming the neural circuits involved in the reward-dependent and motor behaviour (respectively, the ventral tegmental area-accumbens nucleus-prefrontal circuit and the nigrostriatal cortex circuits) [36,37]. Vitamin D provides neuroprotection as well, through several mechanisms including modulation of oxidative stress, regulation of calcium homeostasis and suppression of inflammation [6,30]. This results in an influence on neurocognition, which is also supported by the evidence that VDR and 1-α-hydroxylases are highly expressed in hippocampus and cortex, vital brain areas for the cognitive function like complex planning, processing, and formation of new memories [38].

3. 25(OH)D measurement standardization

The definition of optimal vitamin D status is elusive since there is no evidence-based consensus on which 25(OH)D values define vitamin D insufficiency, deficiency and sufficiency, as shown for other analytes [39–42]. In the absence of such consensus definition, 25(OH)D values below 30 nm/L are considered to be associated with an increased risk of rickets/osteomalacia, while values between 50 and 125 ng/L are considered to be sufficient for skeletal health [43]. What hampers the development of consensus guidelines on 25(OH)D levels defining vitamin D insufficiency is the lack of standardized measurement of serum total 25(OH)D. Standardization processes can align laboratories and assays (within defined statistical limits) with the “true” concentration, based on internationally recognized reference procedures and materials, regardless of the place, time and assay systems. Despite a large amount of research data, the majority of previous studies on vitamin D have reported unstandardized 25(OH)D results; thus, pooling data is difficult, and available meta-analyses are of little use [43]. The vitamin D External Quality Assessment Scheme (DEQAS), which was launched thirty years ago, provides quarterly distributions of five human serum samples to nearly 1000 participants in 56 countries in January 2017 [44]. The main purpose of DEQAS is to assess the accuracy of the results produced by the participants. The accuracy of a result is calculated as the % of bias of the result from “true” result, which is now the target value provided by the National Institute for Standard and Technology (NIST) [45]. When gas chromatography–mass spectrometry (GC–MS) method was regarded as the definitive method for 25(OH)D measurement, the All Laboratory Trimmed Mean (ALTM) was used as the target value, being considered as a good surrogate for the “true” 25(OH)D concentration [46]. In 2013, NIST developed a liquid chromatography-tandem mass spectrometry (LC/MS-MS) assay, which has been accepted by the Joint Committee for Traceability in Laboratory Medicine (JCTLM) as the reference measurement procedure (RMP) for 25(OH)D [47]. With the target value provided by NIST, DEQAS has become an “accuracy-based” scheme. The National Institute of Health Office of Dietary supplements has funded the NIST to produce target values and also funded the vitamin D Standardization Program (VDSP), a collaboration of NIST, DEQAS, Ghent University, the Center for Disease Control and Prevention (CDC), the College of American Pathologist, the International Federation of Clinical Chemistry and Laboratory Medicine, and researchers working worldwide with national health/nutrition surveys [48]. The primary purpose of VDSP is to reduce total

25(OH)D measurement analytical variability, encouraging manufacturers and research and routine clinical care laboratories to use methods and materials traceable to NIST RMPs and standard reference materials (SRMs). Also, VDSP promotes calibration of 25(OH)D results from previous studies with stored sera to the current RMPs. This could allow pooling prior data and producing meaningful meta-analyses, thus facilitating the development of consensus guidelines on vitamin D status. An assay that is traceable to NIST should show precision [coefficient of variation (CV)] ≤ 10% and accuracy (mean bias) ≤ 5% [49]. Recent DEQAS reports show improvement in interlaboratory accuracy, but a substantial sample- to- sample variability remains especially using immunoassays [50]. Main issues with these methods are matrix-effects (due to the presence of other components like lipid in the sample, which compromise the ability of the binding agent to behave identically in the standard and in the sample), and cross-reactivity with other metabolites, like 24,25 (OH)2D [40;44]. The interferences leading to matrix-effect mainly affect automated immunoassays and cannot be overcome by the standardization process. Matrix-effects interferences may occur particularly in samples obtained in pregnant women and hemodialysis, osteoporotic and intensive care unit (ICU) patients [51,52]. On the other hand, LC/MS-MS methods fail to separate metabolites with the same mass of 25(OH)D, as the 3-epi-25(OH)D, leading to overestimating 25(OH)D concentration. 3-epi-25(OH)D can be high in neonates, therefore, clinicians should check that the method used in this population can appropriately separate 25(OH)D and 3-epi-25(OH)D.

The LC/MS-MS method shows the best performance for measuring 25(OH)D in terms of accuracy, compared to immunoassays, which are mainly used by routine clinical care laboratories, yet displaying greater variability than chromatographic assays [53].

The research in the field of vitamin D also focuses on other metabolites and molecules to be used as potential biomarkers for vitamin D status. These include the vitamin D2 and vitamin D3 forms of 1,25(OH)2D, 3-epi-25(OH)D, 24,25 (OH)2D, vitamin D-binding protein (DBP) and free/bioavailable 25(OH)D [40]. NIST has also developed RMPs for 25(OH)D2, and 24R,25(OH)2D3, and disseminated serum-based SRMs with values assigned for 25(OH)D2, 25(OH)D3, 3-epi-25(OH)D3, and 24R,25(OH)2D3 [54].

4. 25(OH)D serum levels in Alzheimer's disease (AD)

A controversy on the association between low 25(OH)D serum levels and the prevalence of cognitive impairment in older adults exists. Although multiple lines of evidence show that vitamin D deficiency is associated with cognitive impairment [55–58], long-term follow up longitudinal studies failed to prove this association [59]. Differences among the results can be explained by several factors, including study populations, heterogeneity of cognitive function measures and cross-sectional design, which sharply limits their power. Importantly, variation of the cut-off used to define vitamin D deficiency actively contributes to discrepancies in the vitamin D literature [7]. Also, limitations of the studies include differences in assay methods and the lack of proved SRMs use (Table 1). Further, interventional studies in this field are few and very heterogeneous, so that findings should be carefully interpreted [60,61]. Taking into account these considerations, the main findings from studies on 25(OH)D serum levels in AD patients can be summarized as follows. Many authors reported an association between low 25(OH)D serum levels and the risk of developing AD [16,55,62–65]. In a large prospective cohort study, Littlejohns [16] measured 25(OH)D serum levels in > 1600 subjects and then evaluated the development of AD in a 5.6 years follow-up period. The authors concluded that the risk of developing AD was significantly higher in participants who were 25(OH)D deficient (deficiency defined as < 50 nm/L). Importantly, the method used to measure 25(OH)D was LC/MS and calibration of serum 25(OH)D concentrations was verified using SRM 972 certified by NIST. In 2017, Licher et al. [64] found that vitamin D deficiency, defined as serum 25(OH)D concentrations below 25 nmol/L, was associated with a higher incidence of AD in 3838 subjects. However, the authors used an electrochemiluminescence binding assay and did not prove to have used RMPs and SRMs. Opposite results were reported by Olsson et al. [66], who analyzed 25(OH)D serum levels by liquid chromatography-mass spectrometry (LC-MS) in 1182 men, finding no association between 25(OH)D levels and the risk of AD. Similarly, Karakis et al. [67] reported no association between vitamin D levels and incidence AD in the Framingham Heart Study participants over a 9 years follow-up period; 25(OH)D measurement in this study was performed by a competitive protein-binding immunoassay. Interventional studies have reported no impact of vitamin D supplementation on AD development, either alone or in combination with other nutrients [68–71], but the effectiveness of the combination of vitamin D and memantine treatment in improving cognition, as compared to memantine alone, has been proved [72]. Balion et al. [73] performed a meta-analysis including 37 studies and 35,000 patients, reporting an association between low 25(OH)D serum levels and risk of AD; however, the authors concluded that differing vitamin D assay methods determined strong heterogeneity among the studies reviewed.

Overall, few studies reported 25(OH)D standardized results and available meta-analysis showed strong discrepancies among the assays systems, thus, no clear evidence on the potential role of 25(OH)D serum levels as a useful biomarker for AD is available.

5. 25(OH)D serum levels in Parkinson's disease (PD)

Low vitamin D serum levels have been reported among PD patients [74–76]. Knekt et al. [77] performed a long-term follow-up longitudinal study in > 3100 subjects, showing that 25(OH)D could predict the risk of developing PD. 25(OH)D was measured by radioimmunoassay, and the use of SRM certified by NIST was reported. However, the small number of PD cases in the sample ($n = 50$) and the possible presence of residual confounders limited the strengths of the study, as stated by the authors. An association between 25(OH)D serum levels and disease severity has also been reported by other authors [78],[79]. Sleeman et al. documented a significant association between serum 25(OH)D concentration and disease motor severity in 145 PD patients who were studied over a follow-up period of 36 months. Although the sample size was relatively small, it should be noted that the samples were analyzed at the MRC Human Nutrition Research in Cambridge, which is part of the VDSP, and quality assurance of 25(OH)D assays was performed as part of the DEQAS [79]. Confirming these results, two recent meta-analyses were performed [80,81], evaluating 2866 (Luo et al.) and 1333 (Zhou et al.) PD patients. Luo et al. concluded that serum vitamin D levels are inversely associated with the risk and severity of PD [80]. However, high heterogeneity was reported among the studies due to the assay methods used, thus, findings from this meta-analysis should be interpreted with caution. Zhou et al. achieved similar results, reporting also no impact of vitamin D supplementation on motor function in PD patients [81].

Taken together, these results might suggest that serum 25(OH)D can be considered as a useful biomarker for PD. Nevertheless, only a few studies evaluating relatively small samples reported standardized data and meta-analysis are of little use, therefore, available data do not support a role for serum 25(OH)D in PD.

6. Conclusions

Although automated immunoassays are commonly used in clinical care laboratories, LC/MS-MS methods should be used to measure 25(OH)D. Literature studies in the field of vitamin D mainly report unstandardized results, which hampers the development of consensus guidelines defining optimal vitamin D status. Collecting and pooling 25(OH)D standardized data and providing meaningful meta-analysis is necessary for this purpose. This could be also important to document

Table 1
Major limitations and strenghts of the observational studies evaluating vitamin D serum levels in Alzheimer's disease patients.

Authors	Year	Limitations	Strenghts	Ref
Littlejohns et al	2014	• Accuracy not reported	• Precision (CV) 3.4% • Vitamin D Deficiency defined (< 50 nmol/L) • Use of LC/MS-MS method • SRM certified by NIST	[19]
Afzal et al.	2014	• Accuracy not reported • Use of immunoassay method • SRM use not reported	• Precision (CV) 10% • Definition of vitamin D deficiency (< 25 nmol/L)	[57]
Buell et al.	2010	• Accuracy not reported Use of immunoassay method • SRM use not reported	• Precision (CV) 9.4% • Definition of vitamin D deficiency (10 ng/mL)	[62]
Feart et al.	2017	• Precision (CV) and accuracy not reported • Use of immunoassay method • SRM use not reported	• Definition of vitamin D deficiency (< 25 nmol/L)	[63]
Licher et al.	2017	• Accuracy not reported • Electro-chemiluminescence binding assay • SRM use not reported	• Precision < 7.8% • Definition of vitamin D deficiency (< 25 nmol/L)	[64]
Olsson et al.	2017	• SRM use not reported • Definition of vitamin D deficiency lacking	• Precision (CV) < 7.6% • HPLC-MS method	[66]
Karakis et al.	2016	• Use of immunoassay method • SRM use not reported	• Precision (CV) < 7.6% • Definition of vitamin D deficiency (10 ng/mL)	[67]

CV: coefficient of variation; NIST: National Institute for Standard and Technology; SRM: standard reference materials.

the role as a biomarker of serum 25(OH)D in AD and PD. Although an association between low 25(OH)D serum levels and the risk of developing these diseases has been reported, few studies presented standardized 25(OH)D data and high heterogeneity among the results exists due to discrepancy among assay methods. To date, literature data do not support the usefulness of 25(OH)D serum levels as a serum biomarker in AD and PD. Further studies using internationally recognized measurement procedures and materials are required.

Declarations of Competing Interest

None.

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