

Pre-Analytical Factors Influence Vitamin D Measurement In Clinical Laboratory

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Abstract:

It has been more than 90 years since the discovery of vitamin D and its capability to cure rickets in children. Recently, awareness is growing of the prevalence of vitamin D deficiency in the general population in association with an increased risk of several diseases. Analyses of vitamin D levels in blood samples provide direct measures of exposure to dietary or supplemented vitamin. This review is focused on the pre-analytical factors and challenges are known to affect the concentrations of vitamin D biomarkers. The pre-analytical measurement of disease relevant vitamin D deficiency in blood can vary as a function of differences in sample handling and processing. It could impact the accurate measurement of the diseases relevant vitamin D deficiency. Majority of the analysis in a clinical biochemistry laboratory requires serum or plasma as the analytical specimen and obtaining serum or plasma from a primary sample is an important step of pre-analytical procedure. Although vitamin D is considered as a relatively stable analyte, effect of pre-analytical conditions and stability of vitamin D in serum and plasma needs to be identified clearly.

Key Words: Vitamin D, Vitamin D binding protein (DBP), Pre-analytical factors, Vitamin D measurements

1. Introduction:

1.1. Vitamin D metabolites measurements in the blood circulation

Vitamin D is a generic name for a heterogeneous group of anti-rachitic substances [1]. It is an essential nutrient of the human diet. There are two biological precursors of vitamin D; the plant origin form is ergocalciferol (D₂) and cholecalciferol (D₃) which is synthesized in the human skin and can be consumed in the form of oily fish, fortified foods, or dietary supplements [1, 2]. The two forms differ in the structures of their side chains, but they are metabolized identically and have equivalent biological activities. Vitamin D is metabolized to several forms, the primary is converted to 25-hydroxyvitamin D (25(OH)D) in the liver which then converted by the enzyme 1 α -hydroxylase to the active form, 1,25-dihydroxyvitamin D(1,25(OH)₂D) [3]. However, 25-OH-D is the predominant circulating form of vitamin D which is considered to be the most reliable index of human's vitamin D status and currently used in clinical practice to assess it [4].

In the circulation, vitamin D like other steroid hormones is bound tightly to a special carrier vitamin D binding protein (DBP) and smaller amounts are bound to blood proteins, albumin and lipoproteins [5]. Only very tiny amounts of the total vitamin D are free and potentially biologically active. It was reported that 85% of vitamin D metabolites are circulated in the blood bounded to DBP, about 15% are loosely bound to serum albumin and only tiny amounts (less than 0.03%) exist in free, unbounded form [6]. The fraction of "bioavailable" vitamin D metabolites consists of the fraction of the free vitamin D and the albumin-bound fraction which then measures around 15% in normal individuals [7]. DBP has the higher binding affinity with 25OHD form than 1,25OHD because the later has an additional hydroxyl group which changes metabolite's molecular geometry [8]. The molar concentration of DBP in blood is more than 20-fold higher compared to the molar concentration of all vitamin D forms [9].

Vitamin D deficiency is pandemic, widespread and is re-emerging as a major health problem globally [10, 11]. A worldwide rapid and dramatic increase in 25OHD assays has been observed over the last years [12, 13]. The assessment of vitamin D is critically important because it plays a major role in calcium and bone metabolism and is essential in cell maturation and proliferation [14]. Deficiency of vitamin D is related to an increased risk of rickets [15], cardiovascular disease [16], colon cancer [17, 18], uterine fibroids [19] and some other diseases such as diabetes type 2 [20], psoriasis[21] and thyroid disease [22], rheumatoid arthritis [23], Alzheimer's disease [24] and Parkinson's disease [25]. Undetected vitamin D deficiency may increase a patient's risk of these diseases. Therefore, the measurement of 25OHD is becoming increasingly important in the management of patients with various disorders and diseases. In addition, due to the widespread of vitamin D deficiency and its potential impact on health, awareness is growing of the need for accurate assessment of vitamin D status in the general population and epidemiological studies. Issues relating to the measurement of vitamin D biomarkers can be broadly divided into three categories; pre-analytical, analytical and post-analytical factors. All these factors may affect the measurement of vitamin D either separately or together. The pre-analytical factors were assessed in the aspects of clinical laboratory and epidemiological studies. This review will concentrate on the common pre-analytical factors may affect the measurement of vitamin D in clinical laboratories.

1.2. Pre-analytical stability of vitamin D

Large scale epidemiological studies designed to evaluate the association between serum or plasma levels of an analyte and risk of disease. These studies frequently require long intervals of storage time between blood collection and laboratory analysis. Thus, the stability of samples under the relevant storage and processing conditions must be known prior to using such samples to test these analytes. Assaying vitamin D is relatively expensive and is therefore often lost in large clinical trials [26]. These assays are extremely variable which confounds the diagnosis of vitamin D insufficiency or deficiency and may produce inaccurate results [27]. This variation was related to the instability of 25OHD or 1, 25OHD in blood or serum exposed to various factors during sample processing, shipping, and storage conditions. Optimization of these conditions is necessary to minimize these pre-analytical errors. Some studies have evaluated the effect of pre-analytical factors on 25OHD or 1,25OHD samples. factors including the influence of temperature, freeze-thaw cycles, centrifugation, tube types and light will be discussed.

1.2.1. Influence of temperature on vitamin D metabolites

There are few studies explored the effect of temperature on the stability of vitamin D metabolites either in whole blood, plasma or serum. The results of these studies were controversy due to some limitations such as small sample size and imprecision of the assay used. A study assessed the stability of different vitamin D metabolites including 1,25OHD and 24,25OHD reported that 1,25OH in serum is stable for 57 days at -80°C , 24h at 4°C , and unstable at room temperature [28]. Similarly, older studies investigated the stability of vitamin D in samples stored at -20°C showed 10% decrease in vitamin D concentration after 3 months [29, 30]. On the other hand, a study evaluated a short term vitamin D stability in serum and plasma samples reported no significant change at 24°C during 72 hours [31]. Moreover, another studies found that 25OHD and 1,25OHD are stable in serum at physiological concentrations at room temperature or under refrigeration for at least 2 weeks [32, 33]. These studies have related this finding to that 25OHD and 1,25OH are extremely stable compounds, as they are bound to vitamin D binding protein (DBP).

1.2.2. Influence of centrifugation

In order to obtain plasma or serum samples for the processing of vitamin D metabolites assays, centrifugation of the blood samples is needed. It is a part of the pre-analytical stage and can be done with or without temperature controlled centrifuges. A recent study which was the first investigated the effect of centrifugation temperature on vitamin D concentration reported that there were no differences between refrigerated centrifuge ($2-8^{\circ}\text{C}$) and centrifuge at room temperature [34]. In addition, some have questioned whether delayed centrifuging reduces vitamin D stability due to hemolysis [35]. Previous studies illustrated that delaying centrifugation of blood samples prior to vitamin D metabolites analysis has no significant differences up to 96 hour after blood collection [36, 37]. These results suggested that vitamin D requires no special care in terms of centrifugation.

1.2.3. Influence of repeated freeze thaw cycles

Prospective studies can store serum from participants which offer the opportunity to further study the relationship between vitamin D deficiency and related diseases. Analyzing large-scale freshly collected samples in one time is impractical and prohibitively expensive. Consequently, these large-scale studies require refreezing and thawing samples more than once which may adversely affect the measurements of the analyte. In this context, no adverse effect was found on the repeated freeze-thawing cycles of vitamin D concentration, although repeated freeze-thaw cycles should be avoided [38, 39].

1.2.4. Influence of tube types used to collect blood samples

Different types of tubes may be used to collect blood sample for analysis of vitamin D metabolites. The available choices of these tubes used are; two different plasma anticoagulant tubes containing either (heparin or EDTA), red top serum tubes or gel tubes. Concerns have been raised regarding using serum or plasma which may affect the concentration of vitamin D measurements [40]. Although there was a little information about the potential impact of blood collection tubes on vitamin D concentrations, conflicted results were reported by two different studies. The first found that sample type did not affect vitamin D stability and both serum and plasma could be used for storage [34], while the second study reported difference in vitamin D concentrations according to tube type in some assays for heparinized plasma [36]. In addition, gel tubes were also used for collecting blood centrifuged to produce serum. This type of tubes contains gel which forms a barrier after centrifugation and has markedly improved serum analyte stability in the primary tube and removed the need for aliquoting the serum [41]. A study by Tanner and colleagues assessed the effects of temperature and time on serum samples collected into gel tubes prior to centrifugation. Overall, most analytes including vitamin D were stable and provided valid results [37]. In contrast, another study found that gels have adverse effects for 25OHD [42]. Hence blood for 25OH D measurement is probably best collected into plain tubes without anticoagulants or gels.

1.2.5. Influence of light

Although protection of specimens from light after blood collection in some studies was recommended for accurate vitamin D analysis [43, 44], the potential impact of light has not been extensively studied. Few reports examined the adverse effect of light on vitamin D metabolites [31, 39, 45]. These studies reported that vitamin D is unaffected by pre-analytical exposure of light experienced in medical laboratories. Moreover, a study evaluated the effect of ultraviolet light on crystalline and serum or plasma 25OHD or 1,25OHD, reported that the UV light destroyed the crystalline 25OHD or 1,25OHD within a few minutes but the serum or plasma 25OHD and 1,25OHD levels did not change after 2 days of UV light exposure. These studies suggested that the stability of vitamin D and its metabolites in serum or plasma when they are insulted with UV light is related to the high bounding affinity of vitamin D to the serum DBP which are resistant to the UV light [46].

2. Conclusion

In large epidemiological studies and large clinical samples, it is not feasible to centrifuge and freeze blood samples immediately after collection. This delay of processing samples may affect the quality of the analytes. The impact of pre-analytical variability on measured vitamin D values is necessary to minimize pre-analytical errors. Although the concentration of vitamin D was reported to be “solid as a rock” under the common pre-analytical conditions experienced in medical laboratories, the routine measurement of vitamin D faces several challenges attributable to the pre-analytical factors. These conditions need to be more investigated and standardized with larger sample size and consider the analytical and post-analytical factors.

3. References

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