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Consensus – reference ranges of vitamin D [25(OH)D] from the Brazilian medical societies. Brazilian Society of Clinical Pathology/Laboratory Medicine (SBPC/ML) and Brazilian Society of Endocrinology and Metabolism (SBEM)

Posicionamento oficial da Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML) e da Sociedade Brasileira de Endocrinologia e Metabologia (SBEM) sobre intervalos de referência da vitamina D [25(OH)D]

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ABSTRACT

Introduction: Vitamin D is considered a pre-hormone and plays a crucial role in calcium homeostasis and, consequently, in bone health. The best source of vitamin D is the skin in response to sunlight. Only small amounts of this vitamin are found in some foods (especially fatty fish), which makes availability of vitamin D in the diet limited. Brazilian population studies show that the prevalence of hypovitaminosis D in our country is high. **Objective:** To define the reference intervals for vitamin D [25(OH)D]. **Discussion:** Consensus of specialists – literature review. **Conclusion:** The standardization of reference intervals is fundamental for the correct diagnosis and treatment of hypovitaminosis D.

Key words: vitamin D; 25-hydroxyvitamin D; reference range.

INTRODUCTION

Vitamin D is considered a pre-hormone and plays a crucial role in calcium homeostasis and, consequently, in bone health. The best source of vitamin D is the skin in response to sunlight. Only small amounts of this vitamin are found in some foods (especially fatty fish), which makes the availability of vitamin D in the diet limited. Brazilian population studies show that the prevalence of hypovitaminosis D in our country is high.

The vitamin D synthesized in the skin or absorbed through dietary intake is transported in blood circulation by a specific protein – vitamin D binding protein (VDBP) – and is metabolized first in the liver and then in the kidney, where it undergoes the first and second hydroxylation, respectively. This physiology is close regulated with the presence of compensatory mechanisms in order to avoid high levels predisposing to toxicity.

Vitamin D deficiency has important clinical effects in bone and muscle, raising the risk for diseases such as osteoporosis and osteomalacia, as well as increasing the risk of falls and fractures.

Data from studies on vitamin D levels in different regions of the world confirm the high prevalence of hypovitaminosis D,

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mainly associated with age over 60 years, high-latitude, winter, hyperpigmentation of the skin, less sunlight exposure, presence of chronic diseases, dietary habits, pregnancy, breastfeeding and absence of foods fortified with vitamin D.

In Brazil, hypovitaminosis D has been documented in several regions of the country, which justifies a critical analysis of its diagnostic criteria. For this purpose, both a correct recommendation for the examination order and the appropriate processing of the sample associated with a critical interpretation/evaluation of the results are essential to prescribe an effective treatment based on the current scientific evidence.

The laboratory determination of the metabolite 25-hydroxyvitamin D [25(OH)D] should be used in the evaluation of an individual's vitamin D status. Assuming that hypovitaminosis D is very prevalent in our country and it has significant clinical importance, this paper aims to discuss and suggest good practice for ordering and interpreting the results, as well as the definition of reference values for 25(OH)D, according to the age group and the presence or absence of chronic diseases. This document is represented by a committee composed of specialists from the Department of Bone Metabolism of the Brazilian Society of Endocrinology and Metabolism [Sociedade Brasileira de Endocrinologia e Metabologia (SBEM)] and the Brazilian Society of Clinical Pathology/Laboratory Medicine [Sociedade Brasileira de Patologia Clínica/Medicina Laboratorial (SBPC/ML)] for the development of recommendations based on scientific evidence available from current literature on vitamin D.

GROUPS AT RISK FOR HYPOVITAMINOSIS D

The main groups at risk for hypovitaminosis D are listed below:

- elderly – over 60 years of age;
- individuals who are not exposed to the sunlight or who have a contraindication to sunlight exposure;
- individuals with recurrent fractures or falls;
- pregnant and breastfeeding women;
- osteoporosis (primary and secondary);
- metabolic bone diseases, such as rickets, osteomalacia and hyperparathyroidism;
- chronic kidney disease;
- malabsorption syndromes, such as after bariatric surgery and inflammatory bowel disease;
- individuals taking medications that may interfere with the formation and degradation of vitamin D, such as antiretroviral, glucocorticoids and anticonvulsants therapy.

RECOMMENDATIONS FOR ORDERING 25(OH)D

- The main clinical recommendations for ordering the 25(OH)D serum level are the diseases or clinical conditions listed above, based on data from clinical history, physical examination, and complementary exams.
- There is no evidence to order the 25(OH)D serum level for the adult population without reported comorbidities. Therefore, indiscriminate population screening is not recommended.

CLINICAL CONSEQUENCES

The most well-known and studied actions of vitamin D are related to bone metabolism, in which its role is crucial. It participates in the intestinal calcium absorption, in the modulation of parathyroid hormone (PTH) secretion, in bone cell and muscle function.

Hypovitaminosis D leads to a deficiency in calcium absorption and thus, causes secondary hyperparathyroidism, which, in turn, may lead to loss of bone mass due to increased resorption and, consequently, to fractures. In situations in which the vitamin D level is extremely low [25(OH)D < 10 ng/ml], a defect in mineralization of bone tissue may occur, characterizing diseases, rickets in children and osteomalacia in adults. Bone pain, muscle weakness, bone deformities and fractures are clinical features of severe vitamin D deficiency. The presence of extraskeletal effects of vitamin D is still under investigation. Meta-analysis with intervention studies suggest that correcting the deficiency reduces general mortality and supplementation would probably has a protective role against cancer, especially in the large intestine. Randomized and placebo-controlled intervention studies are scarce and still unable to demonstrate evidence of many other effects which have been described in other systems. Therefore, supplementation aiming at these other effects is not yet justified.

LABORATORY DIAGNOSIS

The main methods for 25(OH)D measurement are:

- competitive assays using vitamin D binding proteins (VDBP) or anti-25(OH)D antibodies, including radioimmunoassay and enzyme immunoassay, chemiluminescence or electrochemiluminescence

assays. These assays are widely available in clinical laboratories and do not require advanced technology. However, they present several limitations, such as the different reactivity of $25(OH)D_2$ and $25(OH)D_3$ ligands and the incomplete dissociation of 25(OH)D from their binding proteins;

• chromatographic methods such as high-performance liquid chromatography with ultraviolet detection or coupled with tandem mass spectrometry (LC-MS/MS). LC-MS/MS is considered the gold standard for the 25(OH)D measurement because it presents less analytical interference and measures vitamin D directly. However, there are some limitations to its wide use in the clinical laboratory, among them, high cost of equipment, maintenance, specific validations, sample preparation increasing test run time and highly skilled professionals. In addition, it may be subjected to C3-epimer interference, leading to falsely elevated values, especially in children under one year of age.

There is also considerable variation between the 25(OH)D levels obtained in the different methods, hindering the clinical interpretation of the results. The major challenge of the diagnostic market is to ensure a better harmonization between the different tests available, allowing a better comparison of the results between different laboratories. Efforts such as the Vitamin D Standardization Program (EUA) and the Vitamin D External Quality Assessment Scheme (RU) directly target this standardization.

DISCUSSION

The clinical laboratory is responsible for reporting these reference ranges in published reports and should be based on up-to-date literature data, medical society's guidelines and clinical team opinions. There are different ways of defining reference ranges. For 25(OH)D, the evaluation of clinical studies in the literature is currently the best way to define reference intervals, as these studies attempt to investigate the benefits of maintaining certain values in specific populations. However, most of these studies have used, in the past, the Diasorin® radioimmunoassay to measure 25(OH)D, whereas nowadays, most clinical laboratories employ automated, non-isotopic assays for this analysis. Since the correlation between these methods is not so direct, values obtained with the radioimmunoassay may not be transposable to the assays most commonly used today. To suggest reference ranges applicable to all laboratories, the methods would need to be more comparable, however, as described above for vitamin D measurement, there is still considerable variation among the different methods available, generating an additional complicating factor for the definition and the interpretation of these intervals. Although this variation has been reduced in recent years by the standardization programs previously mentioned, the problem of the matrix effect still exists, that is, the interference caused by non-specific endogenous components that vary from sample to sample and can interact with specific immunoassay reagents. This effect may cause unpredictable changes in the results, which can hardly be corrected by the simple standardization of the calibrators of the tests. For this reason, some experts such as Binkley and Carter (2017) suggest that laboratories should apply specific reference intervals, depending on the immunoassay used, in line with what was done with other hormones, or using chromatographic methods by reducing the matrix effect.

REFERENCE RANGES FOR 25(OH)D

Based on literature data, our consensus in relation to the ideal 25(OH)D values for the population should be stratified according to age and individual clinical characteristics:

• above 20 ng/ml – desirable value for healthy population (up to 60 years of age);

• between 30 and 60 ng/ml – recommended value for at-risk groups such as elderly, pregnant, breastfeeding women, patients with rickets/osteomalacia, osteoporosis, patients with a history of falls and fractures, secondary causes of osteoporosis (diseases and medications), hyperparathyroidism, inflammatory diseases, autoimmune diseases, chronic kidney disease and malabsorption syndromes (clinical or post-surgical);

• above 100 ng/ml – risk of toxicity and hypercalemia.

CONCLUSION

This consensus will allow an improved standardization and dissemination to laboratories and physicians regarding the 25(OH)D reference intervals suggested by the medical societies SBPC/ML and SBEM directly involved with the flow of orders, processing, release and interpretation of results of this analyte.

The rate of scientific publications is very dynamic and, should new evidence arise, the societies will make the appropriate updates.

RESUMO

Introdução: *A vitamina D é considerada um pré-hormônio e apresenta papel crucial na homeostase do cálcio e, consequentemente, na saúde óssea. A maior fonte de vitamina D é a pele, em resposta à luz solar. Apenas pequenas quantidades dessa vitamina são encontradas em alguns alimentos (especialmente peixes gordurosos), o que faz com que a disponibilidade da vitamina D na dieta seja limitada. Estudos populacionais brasileiros demonstram que a prevalência da hipovitaminose D no nosso país é elevada.* ***Objetivo:*** *Definição dos intervalos de referência para vitamina D [25(OH)D].* ***Discussão:*** *Consenso de especialistas – revisão da literatura.* ***Conclusão:*** *A padronização dos intervalos de referência é fundamental para o correto diagnóstico e tratamento da hipovitaminose D.*

Unitermos: *vitamina D; 25 hidroxivitamina D; intervalo de referência.*

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