RESEARCH

Vitamin D and PTH: data from a cross-sectional study in an equatorial population

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Abstract

Objective: Investigate the prevalence of vitamin D deficiency in an equatorial population through a large-sample study.

Methods: Cross-sectional study with 30,224 healthy individuals from the North Region, in Brazil (Amazônia – state of Pará), who had 25-hydroxy-vitamin D (25(OH)D) and intact parathyroid hormone (PTH) serum levels measured by immunoassay method. Those with history of acute or chronic diseases were excluded. Abnormal levels of calcium, creatinine, glycemia and albumin were also exclusion criteria.

Results: 25(OH)D levels were 29.1 ± 8.2 ng/mL and values <12.7 ng/mL were equal to < −2 s.D. below average. Hypovitaminosis D was present in 10% of subjects according to the Institute of Medicine (values <20 ng/mL) and in 59%, in consonance with Endocrine Society (values 20–30 ng/mL as insufficiency and <20 ng/mL as deficiency) criteria. Individuals were divided according to four age brackets: children, adolescents, adults and elderly, and their 25(OH)D levels were: 33 ± 9; 28.5 ± 7.4; 28.3 ± 7.7; 29.3 ± 8.5 ng/mL, respectively. All groups differed in 25(OH)D, except adolescents vs adults. Regression model showed BMI, sex, living zone (urban or rural) and age as independent variables to 25(OH)D levels. Comparing subjects with vitamin D deficiency (<20 ng/mL) to those with vitamin D insufficiency (20–30 ng/mL), a difference between PTH levels in these two groups was observed (95.9 ± 24.7 pg/mL vs 44.2 ± 64.5 pg/mL; *P* < 0.01). Additionally, the most accurate predictive vitamin D level for subclinical hyperparathyroidism in ROC curve was 26 ng/mL.

Conclusion: Our equatorial population showed low prevalence of vitamin D hypovitaminosis ranging with age bracket. The insufficient category by Endocrine Society was corroborated by our PTH data.

Key Words
- ▶ epidemiology
- ▶ endocrinology
- ▶ hypovitaminosis D
- ▶ vitamin D

Introduction

The Institute of Medicine (IOM) defines normality of vitamin D as serum 25-hydroxy-vitamin D (25(OH)D) levels above 20 ng/mL, based on the dietary intake needed to meet the requirements for at least 97.5% of the population (1). According to this criterion, several studies worldwide have shown high rates of hypovitaminosis D, with an estimate of 30–40%, in European, Asian, African and South American countries (2, 3, 4). Therefore, hypovitaminosis D has been listed as a public health problem, as one billion people presented 25(OH)D

deficiency (4), with a prevalence ranging from 2 to 90%, depending on cut-off points and selected population (5).

In addition, Manson et al. (6), in 2016, in a re-analysis of IOM data, suggested there was an overestimation in the diagnosis of hypovitaminosis D and believed that sufficiency is reached when serum vitamin D is above 12.5 ng/mL. Bouillon (7), in a review, stated that all guidelines are unanimous in recommending avoidance of 25(OH)D levels below 10 ng/mL, as well as highlighted the controversy involving levels between 10 and 20 ng/mL, which might not indicate deficiency necessarily for the whole population.

Besides the global controversy, large population studies trying to evaluate vitamin D levels in equatorial populations are rare. Little data are available from a small number of locations such as Kenya, DR Congo and Colombia (2, 8). In Brazil, few studies have been carried out to demonstrate the hypovitaminosis D prevalence in healthy subjects. Therewithal, given the country's length, there is considerable climatic difference between the regions and, as far as we are aware, there is no previous study in the Amazonian region. Therefore, it remains unclear whether the current diagnostic criterion for vitamin D deficiency might cause overdiagnosis of this condition. In this context, investigating the prevalence of vitamin D deficiency in an equatorial population, such as ours, through a large-sample study becomes meaningfully important.

Method

Study design and data collection

A cross-sectional study was performed to evaluate serum levels of 25(OH)D, intact PTH and ionized calcium. All our subjects lived in the state of Pará (coordinates: 3.95°S 53.09°W), located in the North Region of Brazil (Fig. 1). Being near the Equator, Pará constantly experiences hot climate and humid weather with little seasonal variation, as do most equatorial areas, which is the reason we did not include seasonality in this study (9). Usual UV index throughout the year ranges from 8 to 10, being classified as 'very high', based on World Health Organization (WHO) criteria (10, 11). Total mean insolation is 2241.6 h/year and total mean irradiation is 5.05 kWh/m^2 day (12, 13). Data were collected from January to December 2019.

A total of 30,224 subjects of both sexes and different age groups who had 25(OH)D serum levels measured at a local laboratory service from January to December of

Figure 1
State of Pará (North Region, Brazil).

2019 were included in the study. All subjects answered a questionnaire; those with previous history of acute or chronic diseases (hypertension, chronic kidney failure, chronic liver disease, autoimmune diseases) were excluded, as well as pregnant women and those taking any medications, including patients who supplemented vitamin D. Subjects who presented abnormal results of serum ionized calcium, creatinine, glycemia and serum albumin were also not eligible (reference values: 1.1–1.3 mmol/L, 0.7–1.3 mg/dL (men) and 0.6–1.1 mg/dL (women), 3.2–4.8 g/dL, respectively). Individuals who had both vitamin D and PTH collected were divided into a smaller group of 1629 healthy individuals and were analyzed separately, in order to properly interpret their data.

Information regarding age, sex, BMI and living zone were collected. Patients were divided according to the age groups defined by WHO, in 2013, as children (0–9 years old), teenagers (10–19 years old), adults (20–59 years old) and elderly (above 60 years old). The impact of seasonality was not evaluated because there are not established patterns of seasons in our region (14, 15). This study was approved by University Hospital João de Barros Barreto Ethics Committee, CAAE number 66977717.8.0000.0017.

Assay

Individuals' blood was collected and, then, serum 25(OH)D was measured quantitatively by the following kit: DiaSorin LIAISON 25-OH-Vitamin D TOTAL

chemiluminescence immunoassay (DiaSorin, Stillwater, MN, USA) (16). DiaSorin LIAISON is one of the methods to evaluate 25(OH)D tested by DEQAS (Vitamin D External Quality Assessment Scheme), the largest specialist external quality assessment (proficiency testing) scheme for the vitamin D metabolites 25(OH)D and 1,25(OH)2D, thus reinforcing the accuracy of our analysis (17). Quantitative serum intact parathyroid hormone (PTH) was determined by electrochemiluminescence (Modular Analytics, E170, Roche). Serum albumin was assessed by enzymatic methodology. Concentration of plasmatic glucose was determined by the hexokinase method. Creatinine was measured through kinetic alkaline picrate assays. Ionized calcium was calculated based on total calcium, protein and albumin.

Statistical analysis

Vitamin D was tested for its normal distribution through the Kolmogorov–Smirnov test. The mean and the s.d. of 25(OH)D levels were calculated. To establish correlations between 25(OH)D levels and variables such as sex, age, BMI and living zone (urban or rural), Pearson or Spearman tests were used. Stepwise multiple regression analysis and simple linear regression were also performed to evaluate the degree of influence of other variables in serum 25(OH)D levels. The SPSS Statistics 22® (IBM Corp.) and RStudio IDE (RStudio, Inc.) software were used for quantitative statistical analysis. The P value <0.05 was considered statistically significant.

As for the subjects dosed for intact PTH, a receiver operating characteristic (ROC) curve was constructed to determine the most accurate vitamin D level to predict subclinical hyperparathyroidism (SHT). The cut-off point with maximum sensitivity and specificity in the ROC curve was defined as the minimum value in the equation $((1 - \text{sensitivity})^2 + (1 - \text{specificity}))$ and the precision was estimated based on the area under the ROC curve.

Results

In our study, 30,224 individuals of both sexes and different age groups were analyzed. The age of the subjects was 40.4±21.4 years, ranging from 0 to 104 (42±20.4 in females vs 36±23.5 in males, $P<0.001$) and BMI was 25.6±5.2 kg/m². Regarding sex, 22,325 (74%) were female and 7899 (26%) were male. They were divided into four age groups: children (0–9 years old), adolescents (10–19), adults (20–59) and elderly (≥60). In our study, there were 3801 (12.6%) children, 2150 (7.1%) adolescents, 18,320 (60.6%) adults and 5953 (19.7%) elderly. 25(OH)D levels were 29.1±8.2 ng/mL and values <12.7 ng/mL were equal to < −2 s.d. below average. All groups differed in age and BMI, as shown in Table 1. Regarding the distribution of vitamin D levels according to each age bracket in our population, all groups differed between one another, except adolescents vs adults (Fig. 2 and Table 1). Furthermore, regarding sex, 25(OH)D levels were higher in males in all groups (Table 2). Only 324 subjects (1%) were below −2 s.d. The median (25th–75th percentile) was 28.3 ng/mL (23.7–33.5). Hypovitaminosis D was present in 10% (3140) of subjects according to the Institute of Medicine (in which abnormal values are those <20 ng/mL) and in 59% (17,847), in consonance with Endocrine Society (which defines values between 20 and 30 ng/mL as insufficiency and values <20 ng/mL as deficiency) criteria. The prevalence of hypovitaminosis D around the world and in our population (according to age groups) is summarized in Tables 3 and 4.

The subjects were from 107 different cities in the state of Pará and those living in urban areas showed lower 25(OH)D levels when compared to those living in rural areas (30.3 vs 28.7 ng/mL, $P<0.001$).

The levels of 25(OH)D correlated with BMI ($r=−0.2$; $P<0.001$) and age ($r=−0.1$; $P<0.01$). Our forward stepwise regression model showed BMI, sex, living zone and age as independent variables to 25(OH)D levels (B=−0.271 (CI: −0.290 to −0.252), $r^2=0.023$, $P<0.001$; B=2.911 (CI: 2.697–3.126), $r^2=0.023$, $P<0.001$; B=−1.763 (CI: −2.057 to −1.468), $r^2=0.005$, $P<0.001$; B=0.019 (CI: 0.014–0.024),

Table 1 Clinical and laboratory characteristics according to age groups.

	Children	Adolescents	Adults	Elderly	
	n=3801	*n*=2150	*n*=18,320	*n*=5953	***P***
Age (years)	6.4 ± 3.3	16 ± 2.3	40.3 ± 10.7	71.1 ± 8.5	<0.001[a]
Sex (F/M) (%)	52/48	66/34	79/21	75/25	<0.001[a]
25(OH)D (ng/mL)	33 ± 9	28.5 ± 7.4	28.3 ± 7.7	29.3 ± 8.5	<0.001[b]
BMI (kg/m²)	19 ± 4.6	22.8 ± 4.7	26.5 ± 4.6	26.8 ± 4.6	<0.001[a]

[a]$P < 0.05$ between all groups. [b]$P < 0.05$ between all age groups except adults vs adolescents ($P = 0.182$).
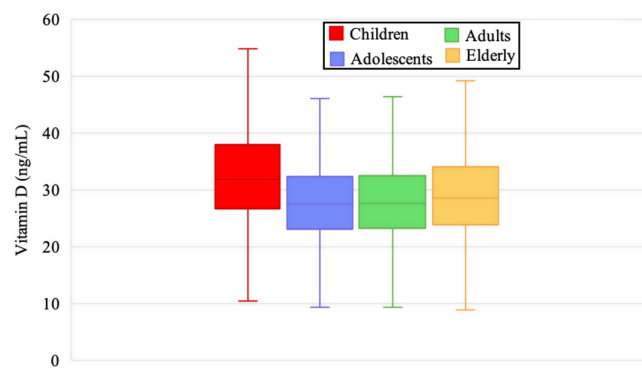
Figure 2
25(OH)D distribution according to age bracket.

$r^2 = 0.002$, $P < 0.001$). These variables showed a poor prediction power, altogether being capable of determining only 5.4% of 25(OH)D levels. An inclination coefficient of -0.235 was obtained using BMI and vitamin D level as variables in a simple linear regression model according to the formula vitamin D level $= 35.002 - (0.235 \times BMI)$, which suggests that, for each BMI unit gained, there would be a decrease of 0.235 ng/mL in vitamin D levels.

The 1692 subjects who had PTH analyzed were divided into two subgroups according to 25(OH)D levels: subgroup 1 (<20 ng/mL) and subgroup 2 (20–30 ng/mL). A difference between PTH levels in these two groups was observed (95.9 ± 24.7 pg/mL vs 44.2 ± 64.5 pg/mL; $P < 0.01$). The proportion of individuals with abnormal PTH, in subjects with vitamin D deficiency or insufficiency according to Endocrine Society, is presented in Fig. 3. In addition, the percentage of functional hypoparathyroidism (hypovitaminosis D with normal PTH levels) among patients with vitamin D insufficiency (20–30 ng/mL) and deficiency (<20 ng/mL) was 91.7% and 77.2%, respectively ($P < 0.01$).

According to the ROC curve, the vitamin D value established as the cut-off point for PTH response to vitamin D insufficiency and, in consequence, for increased risk of bone loss, in our sample was 26 ng/mL, with sensitivity of 54.8%, specificity of 63.2% and accuracy of 58.5%. The ROC curve also presented accuracy of 58.5%, positive likelihood ratio of 1.49 and negative likelihood ratio of 0.72. In addition, we found that in 152 (9%) of our 1692 individuals who had PTH analyzed showed subclinical hyperparathyroidism.

Discussion

Our study showed low prevalence of hypovitaminosis D, ranging with age bracket, when compared to other countries, according to both IOM and Endocrine Society (ES) criteria, even when compared to other equatorial populations. In addition, the insufficient category by ES was corroborated by our PTH data.

Currently, due to laboratory assays and the variability of their cut-off points, 25(OH)D levels above 30 ng/mL are accepted as appropriate by Endocrine Society, once this value would ensure a satisfactory action with no toxicity risk. In 2011, ES defined values between 20 and 30 ng/mL as insufficiency and values <20 ng/mL as deficiency (18). IOM, based on the dietary intake needed to meet the requirements of the population, established vitamin D deficiency as levels <20 ng/mL (19, 20). They also suggest that each population should establish their specific criteria to decide about 25(OH)D supplementation. Nevertheless, Manson *et al.* (6), while assessing data from the National Health and Nutrition Examination Survey (NHANES), suggested <12.5 ng/mL as a cut-off. If Manson's proposition were used in our population, prevalence of vitamin D hypovitaminosis would be extremely low compared to other countries in the equatorial zone (2, 8). Therefore, it could indicate that specific population factors, such as latitude and mean insolation, might be of great importance to determine serum levels of 25(OH)D.

In Brazil, few population studies address this aspect. Unger (21) performed a transversal study in São Paulo, evaluating 603 volunteers aged 18–90 years in 2007. The author found that 77.4% of the participants showed vitamin D insufficiency and 19.3% showed 25(OH)D deficiency. Scalco *et al.*, in a research with 102 noninstitutionalized seniors, found that the prevalence of vitamin D hypovitaminosis was higher (87.5%) (22). Finally, Linhares *et al.* studying 226 children at Recife,

Table 2 25(OH)D levels (ng/mL) according to sex, separated into age groups.

Age/sex	n	Male	Female	P
General	30,224	31.2 ± 8.8	28.3 ± 7.8	<0.001
Children	3801	34 ± 9.3	32 ± 8.7	<0.001
Adolescents	2150	30.1 ± 7.9	27 ± 6.9	<0.001
Adults	18,320	29.8 ± 8.3	27.9 ± 7.5	<0.001
Elderly	5953	32.2 ± 9.1	28.4 ± 8.1	<0.001

Table 3 Prevalence of 25(OH)D deficiency in different countries.

Reference	Country	n	Age range	Prevalence <12 ng/mL (Munns et al. (37))	Prevalence <20 ng/mL (IOM)	Prevalence <30 ng/mL (ES)
Queiroz et al. (2020)	North Brazil	30,224	0–104	0.8%	10.3%	59%
Mogire et al. (2019)	Africa countries	21,474	0–90	18.5%	34.2%	59.5%
Cashman et al. (2016)	Europe	55,844	1–99	13%	40.4%	–
Eloi et al. (2016)	Southeast Brazil (São Paulo)	39,004	2–95	–	33.9%	70.7%
Kiani et al. (2015)	Pakistan	500	1.6–92	–	–	87.6%
Ramnemark et al. (2015)	Northern Sweden	1622	25–74	–	20.8%	–
Gill et al. (2014)	Northeast Australia	2413	24–95	–	22.7%	–
Unger et al. (2010)	Southeast Brazil (São Paulo)	603	18–90	–	–	78%

ES, Endocrine Society; IOM, Institute of Medicine.

Brazil, did not find cases of vitamin D deficiency (23). All those studies used Endocrine Society parameters of normality. The first two studies occurred in the South-Southeast region of Brazil (22°–33° S), with lower mean insolation, which could justify the higher hypovitaminosis D prevalence compared to our data. The third study, which evaluated just a selected children population, found no case of hypovitaminosis D and occurred in the Northeast region of Brazil, in a city with latitude close to ours. Those last findings suggest that latitude, mean insolation and age must be considered when establishing normal vitamin D values in a population, which reinforces our findings about age bracket influence on vitamin D.

In addition, we found higher levels of 25(OH)D in males. One possible explanation would be a greater sun exposure of the thorax – which is a region of better absorption of 25(OH)D. Our results are in agreement with the findings of Al-Ghamdi (24) and Kiani et al. (25). The former evaluated 150 subjects from Saudi Arabia and observed vitamin D lower in girls, probably because of their dressing costumes. In Amazonia, due to

the hot weather, men usually dress shirtless and, consequently, have a higher thorax exposure. Another hypothesis would be the influence of testosterone levels. It has been suggested that testosterone levels could be associated with higher 25(OH)D levels (26, 27, 28, 29). In fact, Araujo et al. also described vitamin D levels higher in males than in females, also suggesting this hormonal influence (30). A possible mechanism that could explain this association is not well established.

Our data also showed a decrease in circulating concentrations of serum 25(OH)D while BMI increased, which indicates that poor vitamin D levels are linked with higher BMI. The proposed mechanisms to explain this include a lack of sun exposure, modified vitamin D

Table 4 Prevalence of vitamin D deficiency in our population according to different criteria.

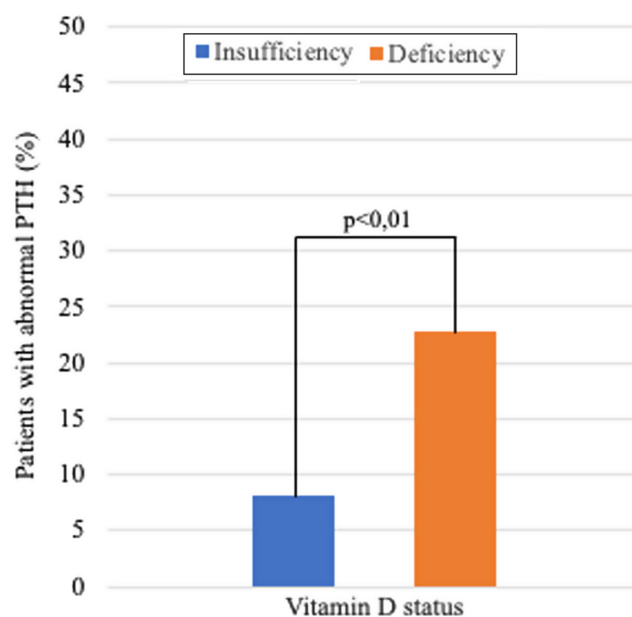| Vitamin D cut-offs (ng/mL)	<12 (Munns et al. (56))	<20 (IOM)	<30 (ES)
Children (%) n=3801	0.1	4.5	40.9
Adolescents (%) n=2150	0.8	12.2	64.2
Adults (%) n=18,320	0.9	11.3	62.9
Elderly (%) n=5953	1.1	10.5	57.0
Total (%) n=30,224	0.8	10.4	59.0

Figure 3
Distribution of patients with secondary hyperparathyroidism according to vitamin D status (Endocrine Society).

activation and increased vitamin D storage in adipose tissue (18, 31, 32). Indeed, obese individuals expose themselves less to solar UV radiation, diminishing cutaneous synthesis of vitamin D3 from 7-dehydrocholesterol (32). It was showed, in a study with obese patients, a higher prevalence of hypovitaminosis in this group, as none of them had the habit of sunbathing. Their clothes used to cover as much skin as possible, generating the hypothesis that low concentrations of vitamin D may be related to low sun exposure in those patients (32).

Eloi *et al.* (33), in a similar research in São Paulo, based on the ES, performed an investigation in patients aged from 2 to 95 years, and observed vitamin D concentrations <20 ng/mL in 33.9% of the patients, presenting higher vitamin D levels in summer (38.4%) when compared to winter (23.3%). In our study, it was not possible to assess this issue, due to the lack of proper season distinction in the state of Pará, which is situated near the Equator line presenting higher insolation throughout the year.

Our study showed that people living in rural areas have higher vitamin D levels. This finding agrees with a meta-analysis that took place in African countries recently (2). However, it remains a controversial topic. Fang *et al.* (34), in a study based in China, with 1814 subjects, showed slightly higher vitamin D levels among urban residents compared to rural ones, but also pointed out that its results are opposed to many similar researches in Asia (35, 36, 37, 38). Similarly, an Indian study demonstrated lower 25(OH)D levels in rural subjects despite plentiful sun exposure (39). In our region, the dressing habits of countryside inhabitants, such as using lighter and fewer clothes, and the fact that rural areas have less impairment of sun exposure due to shorter buildings could influence our results. In fact, KimLi *et al.* (40) established skin exposure as the single strongest contributor to the explained variance in 25(OH)D. However, this issue needs to be better analyzed.

The variables studied in our regression model showed a low predictive power, being capable of determining only 5.4% of 25(OH)D levels. An important hypothesis that could explain these findings is the assessment of polymorphism consequences for the vitamin D receptor (VDR) function. This individual genetic diversity could play a central role in determining 25(OH)D levels (41, 42, 43, 44). According to Nissen (45), who analyzed twenty-five different genetic variants in seven different genes, it was concluded that polymorphisms in the *CYP2R1* and *GC* genes are associated with serum variations in the vitamin D. In this same perspective, Husain *et al.* (46), who compared Americans with European and African ancestry

with similar lifestyles and demographic conditions, demonstrated that those with African ancestry had lower 25(OH)D values. Both studies suggest that specific ethnic and genetic determinants may influence vitamin D levels. In Brazil, the expressive miscegenation makes ethnic analysis difficult. This could explain the difference between hypovitaminosis D prevalence in our study when compared to those from the rest of the world.

Analyzing the ROC curve in this study, we found that vitamin D level of 26 ng/mL is the best cut-off point for the PTH response to vitamin D insufficiency and, consequently, for increased risk of bone loss. It suggests that subjects with vitamin D levels lower than 26 ng/mL could have an additional benefit in supplementation, since a large number of people who fit in this condition are asymptomatic to SHP. According to ES definitions, this value is classified as insufficiency, reinforcing that this group of individuals should receive special attention in clinical practice.

Another aspect that should be addressed is the method for assessing vitamin D. Liquid chromatography–mass spectrometry is considered the gold standard for measuring 25(OH)D. It has the advantage of allowing sample adaptation and high specificity; however, it is a complex, expensive and rarely available assay (47). The automated immunoassay is the most widely used method globally, standardized in clinical practice, as it is easier to perform, faster and less costly (47, 48). In our study, aiming at the clinical applicability of the findings, the automated immunoassay was used.

In Brazil, low vitamin D intake is commonly observed. Filgueiras *et al.* (49) found an elevated prevalence of inadequate vitamin D intake (91.3 %) among 378 children. Peters *et al.* (50), studying 136 adolescents, and Cembranel *et al.* (51), assessing 1051 patients between 22 and 63 years, described that 85.1% and 100% of subjects did not meet the daily adequate intake recommendation of vitamin D. In 2013, a National Dietary Survey with Brazilian elderly stated that our region had more adequate levels of vitamin D intake when compared to other regions of Brazil, even though inadequacy was still very present (52). This might contribute to the lower prevalence of vitamin D hypovitaminosis in our population.

Skin pigmentation also might have an influence in vitamin D levels. Libon *et al.* (53), in a research with Fitzpatrick skin types II, III (fair-skinned) and VI (black-skinned) individuals, found that after a single total body UVB exposure fair-skinned people presented higher levels of vitamin D when compared to black-skinned people. In agreement, a systematic review conducted by

Xiang *et al.* (54) concludes that vitamin D production was less effective in those with pigmented skin. In the North region of Brazil, black people correspond solely to 6.5% of the population (55). This also might have influenced our results.

In fact, as a limitation of our study, we did not consider the amount of time of exposure to sunlight, skin type and vitamin D daily dietary intake, which are known as important factors impacting serum vitamin D levels.

Finally, the low prevalence of hypovitaminosis D in our population was established by IOM criteria of 20 ng/mL, using data from our 30,224 subjects who performed 25(OH)D serum dosage. On the other hand, the value of 26 ng/mL resulted from a ROC curve constructed to determine the most accurate vitamin D level to predict subclinical hyperparathyroidism, which should be considered for treatment in risk groups for bone mass loss, for example. However, it would not be a general population cut-off point.

Conclusion

Our equatorial population showed low prevalence of vitamin D hypovitaminosis, according to both IOM (<20 ng/mL) and ES criteria (<30 ng/mL), ranging with age bracket. The insufficient category by Endocrine Society was corroborated by our PTH data. Since our regression model could only determine 5.4% of the vitamin D levels, the individual characteristics of each subject should be taken into account to establish inadequate levels of vitamin D.

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