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Optimal vitamin D status and its relationship with bone and mineral metabolism in Hong Kong Chinese

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ABSTRACT

Background: Although 25-hydroxyvitamin D (25[OH]D) is commonly used to define vitamin D status, there is no consensus on the cutoff levels for vitamin D deficiency and insufficiency. In this study, we aimed to identify the 25(OH)D threshold that maximally suppressed parathyroid hormone (PTH) in Hong Kong Chinese population.

Methods: The study included 5,276 participants (70% female) of the Hong Kong Osteoporosis Study aged 20 or above who had total 25(OH)D measured. Three-phase segmented regression was used to identify the optimal break-point between 25(OH)D and PTH.

Results: The prevalence of vitamin D deficiency observed was 43.8% and the prevalence of insufficient (<75nmol/L) or deficient (<50nmol/L) vitamin D levels was 90.1% in our study population. Using unadjusted three-phase segmented regression, the estimated first and second break-point of 25(OH)D on PTH suppression were 32nmol/l (95% CI:29-35) and 89nmol/L (95% CI:77-101) with an r^2 of 0.048, whereas the estimated first and second break-point of 25(OH)D were 27nmol/L (95% CI:24-30) and 47nmol/L (95% CI:37-56) after adjusting for factors affecting bone and mineral metabolism. In addition, the relationship between 25(OH)D and PTH significantly differed by sex and age.

Conclusion: The threshold for 25OHD at the point of maximal suppression of PTH estimated in this study was lower than the suggested threshold of vitamin D deficiency in the literature,

perhaps due to race or assay differences, and the relationship between vitamin D and PTH changed with sex and age. Standardization in the methodology of searching for the optimal break-point is desirable so that a consensus on cutoff points can be obtained.

INTRODUCTION

Vitamin D is a fat-soluble vitamin that is essential for human health. Sun exposure is the major source of vitamin D, while other sources include diet and supplementation [1, 2]. The circulating form of vitamin D is 25-hydroxyvitamin D [25(OH)D], while the active form is 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], which is involved in gene expression regulation and hence cellular function [3, 4]. Vitamin D is well recognized for its importance to the musculoskeletal system, especially in regulating bone metabolism and calcium homeostasis. Recent studies have shown that vitamin D is also associated with extra-musculoskeletal conditions, such as autoimmune diseases, cancers, and metabolic and cardiovascular diseases [2, 5, 6].

Vitamin D deficiency is highly prevalent as it affects over one billion people worldwide [1], and so it is considered a major public health problem. Currently, the two most widely used cutoff points for vitamin D insufficiency and deficiency are from the Institute of Medicine (IOM) and the Endocrine Society. They define vitamin D deficiency and insufficiency as circulating 25(OH)D <30nmol/L and 30nmol/L-50nmol/L [7], or circulating 25(OH)D ≤50nmol/L and 50nmol/L-75nmol/L [8], respectively. The conversion factor for concentration unit from nmol/L to ng/ml is 0.4. These proposed cutoff points were based on the assessment of the optimal serum concentration of 25(OH)D associated with various health outcomes, such as, for IOM, bone health. However, there is so far no

consensus on the optimal cutoff points for vitamin D deficiency. Since most studies were performed in Caucasians, whether the suggested cutoff points are applicable in other populations are controversial, especially in those populations with darker skin and different lifestyles, such as African and Asian [9].

The majority of the population in Hong Kong are Southern Chinese, who have darker skin and a different food consumption pattern when compared to Caucasians. In this study, we aimed to estimate the prevalence of vitamin D deficiency and insufficiency and to evaluate the break-point that maximally changes the slope for the association between vitamin D and parathyroid hormone (PTH) in the Hong Kong Chinese population.

METHOD

Subjects

The Hong Kong Osteoporosis Study is a prospective follow-up study on musculoskeletal and mineral metabolism related conditions. The details of this study have been described elsewhere [10, 11]. In brief, the baseline examinations were carried out between 1995 and 2010. Participants were all Hong Kong residents of self-reported Southern Chinese descent recruited from health fairs and public roadshows. After providing written informed consent, the participants were interviewed, underwent clinical examination and gave a blood sample. Demographic data were collected on anthropometric measurements, socioeconomic status, education level and medical and reproductive history by trained

interviewers using a structured questionnaire. Lifestyle information such as smoking status, drinking habit and physical activities were also obtained. In the current study, we included participants from the Hong Kong Osteoporosis Study aged 20 or above with total 25(OH)D measured and excluded those with known skeletal disease (including primary hyperparathyroidism) or prescription of medication that may affect bone metabolism (N=6,104). Of those, participants with missing variables in the fully adjusted model (body mass index [BMI, N=16]; smoking status [N=63]; drinking status [N=65]; sport habit [N=63]; serum calcium [N=34]; albumin-corrected calcium [N=251]; phosphate [N=59]; alkaline phosphatase [ALP, N=69]; PTH [N=391]; lumbar spine bone mineral density (BMD) Z-score [N=353]; femoral neck BMD Z-score [N=269]) were excluded from the analysis. In total, 5,276 participants, 1,583 males (30%) and 3,693 females (70%), were included in the final analysis.

BMD Measurement

BMD at the spine L1-L4, femoral neck, and total hip were measured using dual-energy X-Ray absorptiometry (DXA; Hologic QDR 4500 plus). Daily calibration of the equipment was performed and the in vivo precisions of DXA measurement at the lumbar spine, FN, and total hip were 1.2%, 1.5% and 1.8% respectively. All measurements were performed by a licensed medical technologist according to standard procedures and the equipment manufacturer's instructions. BMD measurements are shown in absolute value (gram per squared-centimeter). The age- and sex-matched Z-score was used to compare BMD, due to the large age range of the subjects.

Laboratory analyses

Serum intact PTH was measured by chemiluminometric assay (Chiron Diagnostic Corporation, USA). The inter-assay coefficient of variation was less than 10% while the intra-assay coefficient of variation was less than 11.8%. Serum 25(OH)D levels were measured by direct enzymatic immunoassay EIA (IDS Ltd, UK). The minimum detectable dose of 25(OH)D was 4.8ng/mL, and the coefficient of variation for assay precision was less than 10%. Quality controls were included in each batch of assay.

Statistical analysis

The baseline characteristics of the study subjects are shown as means and standard deviations stratified by serum 25(OH)D status. Variables with a skewed distribution were log-transformed before analysis. Serum 25(OH)D level was analyzed as a continuous variable and as a categorical variable, stratified by the clinical cutoff points for deficiency (sufficient (>75mol/L), insufficient (≤75 and >50nmol/L) and deficient ≤50ng/ml) suggested by the Endocrine Society to reflect the association of 25(OH)D not only with bone health outcome but also with non-skeletal health outcome. The relationship between vitamin D and bone and mineral biomarkers were first evaluated using locally weighted regression and scatterplot smoothing (LOESS) technique. To evaluate the break-point that maximally changed the slope in the association between vitamin D and PTH, segmented regression

was carried out using R package “segmented”. As segmented regression can be affected by outliers, we excluded participants with serum 25(OH)D above the top 0.5^{th} or below the lowest 0.5^{th} percentile which were referred as extreme outliers. In the R package “segmented”, the algorithm searched along the total 25(OH)D iteratively and identified the break-point with the lowest overall residual mean standard error. Three-phase model was performed based on the findings in LOESS and literature, which showed a better fit than a two-phase model [12]. The LOESS and segmented regression were performed for all participants and separately for sex and age group [13]. We also performed a fully adjusted segmented regression to estimate the break-point by adjusting for age, sex, BMI, smoking status, drinking status, sport habit, serum calcium, phosphate, ALP, lumbar spine BMD Z-score, and femoral neck BMD Z-score. To investigate any effect of dietary calcium intake and the pathologic condition of renal function on the breakpoint estimation, we stratified the study population based on the dietary calcium intake levels assessed by food frequency questionnaires (FFQs) [14] and the estimated glomerular filtration rate (eGFR) of 60 mL/min/1.73m^2 was used as cut point in additional sub-group analysis. The eGFR was estimated using the Chinese-MDRD equation. Model fit was expressed as r^2 for comparison between the regression models [15, 16]. All statistical analysis was done using SPSS (V24, IBM Corp) or R software (V3.1.2).

RESULTS

Characteristics of the subjects

Characteristics of the participants stratified by vitamin D status are shown in Table 1. The prevalence of vitamin D deficiency and insufficiency were 43.8% and 46.3% in our study population. Vitamin D deficiency was more prevalent in the young age group than the middle age group (from 62.5% in 20's to 32.8% in 50's) and the elderly (35% in 60's and 44.5% in 70's or above). Serum vitamin D was positively correlated with serum calcium, ALP, and femoral neck BMD Z-score and inversely correlated with PTH and phosphate. After adjusting for sex, age, season, BMI, drinking and smoking status, and physical activities, the correlation of serum 25(OH)D with phosphate and ALP became insignificant.

Determination of cut point of optimal 25(OH)D, using bone health as selected health outcome in Hong Kong Chinese

Relationships between 25(OH)D, PTH, and other bone and mineral markers were evaluated using LOESS regression plots (Figure 1 and supplementary figure 1). Based on segmented regression analysis, the break-points are shown in Table 2. In all participants, the first and second break-point estimated was 32nmol/L and 89nmol/L, respectively. In the fully adjusted model, the first and second break-point were estimated to be 27nmol/L (95% CI: 24 to 30) and 47nmol/L (95% CI: 37 to 56), respectively. Notably, the slope after the break-point was generally less steep compared to the slope before the break-point; for example, the first, second, and third slope in all participants group was -0.104 (95% CI: -0.148 to -0.06); -0.02 (95% CI: -0.024 to -0.016), and 0.011 (95% CI: -0.007 to

0.028), respectively, whereas the slope was significantly different before and after the break-point (the superimposed graphs of LOESS are provided in supplementary figure 2). The correlation between PTH and 25(OH)D significantly differed ($P<0.05$) by sex and by age groups (Age 20-59 vs. Age ≥60). The estimated first and second break-point of each subgroups are provided in Table 2. In general, the range of the first and second break-point fell in the range of 30-35 and 71-97, respectively. When compared with a two-phase segmented regression model, three-phase segmented regression model had higher r^2 value values (supplementary table 1). In additional subgroup analysis on the effects of dietary calcium intake and renal function, the estimated breakpoints were similar (¬32nmol/L and ¬89nmol/L for the first and second breakpoint respectively) (supplementary table 2 and supplementary figure 3). This suggests that the current findings are less likely affected by calcium intake and similar breakpoints are found in the subjects with normal renal function. In addition, we had performed analysis for the estimation of the breakpoints of 25(OH)OD on BMD at the lumbar spine and femoral neck but we did not observe any breakpoints of significance. This suggests that there was no association of 25(OH)D with BMD as observed in LOESS plots (data not shown).

DISCUSSION

This study showed that vitamin D insufficiency or deficiency is highly prevalent (90.1%) in Southern Chinese, whereas the vitamin D deficiency cutoff point suggested by the Endocrine Society (50nmol/L) is substantially different from the estimated first break-point (32nmol/L) of 25(OH)D on

PTH in our Chinese population. Moreover, the estimated break-point of 25(OH)D varied with sex and age, and after adjusting for confounding factors.

Prevalence of vitamin D deficiency and sufficiency in Chinese varied greatly among studies. Previous studies reported the prevalence of vitamin D deficiency in Urumqi (43.5°N) [17], Beijing (39.5°N) [17], Lanzhou (36°N) [18], Shanghai (31°N) [19, 20] as 66.8%, 73.5%, 70%, and 30-69.2%, respectively. Discrepancy in vitamin D status observed could be due to differences in study design, such as different age groups, disease status as well as difference in latitude [21]. In Guangzhou [17], a region at a similar latitude as Hong Kong (22°N), the prevalence of vitamin D deficiency is comparable (39.6% in Guangzhou vs. 43% in Hong Kong).

Although a higher prevalence of vitamin D deficiency and insufficiency was observed in younger participants in our study, the availability of 7-dehydrocholesterol, the vitamin D precursor in skin, correlated negatively with age [22]. Our findings are indeed in agreement with previous studies showing lower vitamin D levels in younger people [21, 23, 24]. Since serum vitamin D level is determined by many factors, this finding could be due to lifestyle in the young population, such as avoidance from sun exposure, use of sunscreen products and cover-up clothing, and the preference of indoor activities. The findings in the Macau population also showed that “sitting” was a risk factor for vitamin D deficiency, suggesting that long working hours sitting in office or other indoor space might

contribute to this observation.

The prevalence of vitamin D deficiency and insufficiency is affected by the cutoff values. Most studies used PTH as a target to evaluate the optimal cutoff point of vitamin D, as low 25(OH)D level leads to elevation in PTH (secondary hyperparathyroidism), which increases bone loss and risk of fractures [25-27]. However, a wide range of optimal cutoff points for 25(OH)D deficiency has been identified, ranging from 10ng/ml (~25nmol/L) to 50ng/ml (~125nmol/L), and these inconsistent findings might have been affected by race, age group, sex, assays, and statistical methods used in the studies [15]. Nevertheless, we showed that a three-phase segmented regression model has a better model fit than a two-phase or linear regression model. This is in agreement with a previous study showing that the three-phase model appeared to be superior to the two-phase model in describing the relationship between 25(OH)D and PTH [12]. Using a three-phase model, two break-points were identified at 32nmol/L and 89nmol/L when all data were analyzed together. For other subgroups, the first break-points suggested were generally between 30 and 35nmol/L; whereas the second break-points were generally between 71 and 97nmol/L.

It is commonly agreed that vitamin D status can be defined as “deficiency”, “insufficiency”, and “sufficiency”. In the current study, we demonstrated that the relationship between serum PTH and 25(OH)D has three phases. In the first phase (before the first breakpoint), PTH was suppressed

maximally and decreased rapidly with increasing 25(OH)D (a steep slope). This range may be defined as vitamin D deficiency. In the second phase (between the first and second breakpoint), PTH was further suppressed but the association of 25(OH)D with PTH was less than in the first phase (a less steep slope). This range may be defined as vitamin D insufficiency. In the third phase (after the second breakpoint), the association of 25(OH)D with PTH has changed significantly to a nearly flat relationship. This may represent the status of vitamin D sufficiency, where vitamin D has no longer any significant association with PTH.

Our finding was based on a general population model like the IOM [28], and the first break-point we found (32nmol/L) is similar to the vitamin D deficiency threshold suggested by the IOM (30nmol/L). Interestingly, in the fully adjusted model, the first (27nmol/L) and second (47nmol/L) break-points were similar to the threshold of vitamin D deficiency and insufficiency suggested by the IOM (30nmol/L and 50nmol/L, respectively). Some authors reported a plateau after the break-point [29-32]; however, we first observed a less steep inverse relationship of PTH with 25(OH)D after the first break-point, and then followed by a plateau after the second break-point. This is in agreement with a recent study in Shanghai, which showed a steep decrease in PTH up to 20ng/ml of 25(OH)D then followed by a gradual decrease in PTH after 20ng/ml (~50nmol/L) with a plateau [20].

In the current study, we found that the break-point would change if a different adjustment model was

used, thus highlighting the need to standardize the methodology of searching for the optimal break-point between 25(OH)D and PTH [12]. Interesting, in the fully adjustment model, the second break-point was affected more significantly in magnitude (from 89 to 47nmol/L) than the first break-point (from 32 to 27nmol/L). These may suggest that the confounders may have larger effects on vitamin D levels in the second phase than in the first phase. During the first phase (~vitamin D deficiency), PTH may be the major determinant of serum levels of 25(OH)D, and so 25(OH)D was less affected by other factors; on the other hand, when the serum levels of 25(OH)D go up, the effect of PTH becomes less (as reflected by the estimate in the regression model), while the effect of other factors become larger. To evaluate if this hypothesis is true, we evaluated the beta of the confounders during the first and second phases and found that the estimates of these confounders were generally larger in the second phase than in the first phase (data not shown). These findings explain why the adjustments had larger effects on the insufficient level than the deficient level.

To define vitamin D deficiency, we may need to consider age and sex. There seemed to be a sex-specific (supplementary figure 2a), and age-specific (supplementary figure 2b) relationship between 25(OH)D and PTH. Sex-specific association of vitamin D on health has been well documented in the literature [33-35]. Our study suggested that the relationship of 25(OH)D and PTH was different between sexes, with a steeper slope in men than in women, which is opposite to a report that the negative correlation coefficient was higher in women than in men [34]. The relationship

between 25(OH)D and PTH is complex and modulated by a number of factors, such as estrogen and FGF23 [36] that are higher in women than in men [37]. More studies are required to investigate this difference in regulation of PTH by vitamin D. On the other hand, a recent cross-sectional study on 25(OH)D and PTH in 312,962 subjects showed that age (age<20; age 20 to 39; age 40 to 59; and age≥60) has a strong effect on the relationship between 25(OH)D and PTH [13]. Using the same age group cutoffs, we also showed that the relationship of 25(OH)D with PTH was significantly different between age≥60 and age<60, however we did not see a significant difference between the age groups of 20-39 and 40-59.

Our study has several strengths. The study population is large and well characterized with multiple markers of bone and mineral metabolism. Nevertheless, there are limitations. First, 25(OH)D was measured using ELISA instead of a more accurate LC/MS method and the assay used was not part of the Vitamin D Standardization Program (VDSP). Nevertheless, it has been demonstrated that the correlation between these two methods is excellent in healthy subjects (R>0.93) [38]. Second, we measured total ALP instead of the bone isoform of ALP. We previously showed that the regulation of ALP and bone-specific ALP was different [39], thus the relationship between 25(OH)D and bone-specific ALP remains to be studied. Third, detailed vitamin D supplementation data were not available. Fourth, over 70% of our study subjects were female and this is not so in the general population.

In conclusion, the estimated prevalence of vitamin D deficiency and insufficiency is high in Hong Kong Chinese. In addition, different statistical models may lead to different findings, while three-phase segmented regression is a more appropriate model for the analysis. The first and second break-point of vitamin D on PTH is estimated to be 32nmol/L and 89nmol/L before adjustment and 27nmol/L and 47nmol/L after adjustment in Hong Kong Chinese. These findings demonstrate the importance of the statistical methods used in the analysis. Furthermore, the effect of PTH on 25(OH)D is higher during the first phase, than in the second or the third phases, as reflected by the estimates. This demonstrates why they are proposed cutoff points on vitamin D deficiency and insufficiency. However, standardization in the methodology of searching for the optimal break-point is desirable so that a consensus on the cutoff point can be obtained.

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