Vitamin D and Its Relationship with Markers of Bone Metabolism in Healthy Asian Women

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Background: In this study, we aimed to determine the normal ranges of 25-hydroxy-vitamin D₃ (25-OHD₃), parathyroid hormone (PTH), and the markers of bone turnover, procollagen type 1 N propeptide (P1NP) and C-terminal cross-linked telopeptide of type 1 collagen (CTX), in normal healthy women in Singapore, and to explore the relationship between vitamin D, PTH, and these markers of bone turnover in the women. **Methods:** One hundred and ninety-seven healthy women, aged 25 to 60, were selected from a hospital staff health screening program; 68% were Chinese, 18% Malay, and 14% Indian. P1NP, CTX, and 25-OHD₃ were measured using the Roche Cobas[®] electrochemiluminescence immunoassay. Serum PTH was measured using the Siemens ADVIA Centaur[®] immunoassay. **Results:** Sixty-five percent had 25-OHD₃ concentrations <50 nmol/l. Vitamin D insufficiency (25-OHD₃ < 50 nmol/l)

was more prevalent in Malays (89%) and Indians (82%) compared to Chinese (56%). There was no correlation between vitamin D and age. PTH positively correlated with age, and Malays and Indians had higher PTH concentrations than Chinese. There was an inverse correlation between PTH and 25-OHD₃, but no threshold of 25-OHD₃ concentrations at which PTH plateaued. The bone turnover markers P1NP and CTX inversely correlated with age but were not different between ethnic groups. CTX and P1NP exhibited good correlation, however, there was no significant correlation between 25-OHD₃ or PTH concentrations and the bone turnover markers P1NP and CTX. **Conclusions:** Healthy women in Singapore have a high prevalence of vitamin D insufficiency. Vitamin D insufficiency was more prevalent in Malays and Indians compared to Chinese. J. Clin. Lab. Anal. 27:301–304, 2013. © 2013 Wiley Periodicals, Inc.

Key words: 25-OHD₃; PTH; P1NP; CTX; association; healthy Asian women

Many common disorders, such as cancer, diabetes, cardiovascular disease, infectious and autoimmune disease, as well as increased risk of mortality, have been found to be associated with low vitamin D concentrations (1). Vitamin D status is most reliably determined by assay of serum 25-hydroxyvitamin D (25-OHD). The Endocrine Society Task Force Clinical Practice Guideline (2011) defined vitamin D deficiency as a 25-OHD concentration below 50 nmol/l (20 ng/ml) and vitamin D insufficiency as 25-OHD between 50 and 75 nmol/l (21–29 ng/ml) (2). The Institute of Medicine Report (2011) on Dietary Reference Intakes for Calcium and Vitamin D concluded that a 25-OHD concentration of 40 nmol/l (16 ng/ml) meets the needs of 50% of the general population while a 25-OHD concentration of 50 nmol/l (20 ng/ml) meets the needs of 97.5% of the general population (3). Vitamin D insufficiency, with 25-OHD concentrations between 25–75 nmol/l (10–29 ng/ml), is prevalent in many countries around the world (4).

Vitamin D deficiency impairs calcium and phosphate absorption, resulting in poor mineralization of bone, and is associated with secondary hyperparathyroidism (5). Many studies have reported an inverse relationship between vitamin D and parathyroid hormone (PTH) concentrations, where PTH plateaued at concentrations of 25-OHD between 75 and 100 nmol/l (30–40 ng/ml) (6).

Biochemical markers of bone metabolism are increasingly used in the management of patients with osteoporosis and other metabolic bone diseases. They provide

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Received 19 December 2012; Accepted 7 February 2013

DOI 10.1002/jcla.21602

Published online in Wiley Online Library (wileyonlinelibrary.com).

information on fracture risk independently from bone mineral density (BMD) (7). The International Osteoporosis Foundation (IOF) and the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Working Group on Bone Marker Standards recommend serum procollagen type 1 N propeptide (PINP) as the reference bone formation marker and serum C-terminal cross-linked telopeptide of type 1 collagen (CTX) as the reference bone resorption marker for international standardization (7).

The aims of this cross-sectional, observational study were to determine the normal ranges of vitamin D, PTH, and the markers of bone turnover, PINP and CTX, in normal healthy women in Singapore, and to explore the relationship between vitamin D, PTH, and these markers of bone turnover in the women.

One hundred and ninety-seven nonpregnant female hospital staff from 25 to 60 years of age, were recruited as part of a health screening exercise. The median age was 38. The ethnic representation was 68% Chinese, 18% Malay, and 14% Indian. Informed consent was obtained from all participants.

Fasting blood samples were collected in the morning and serum was used for measurement of biological markers. Serum 25-OHD₃ was measured using the Elecsys[®] 25-OHD₃ serum assay (Roche Diagnostics, GmbH, Germany). Serum intact PTH was measured using the Siemens ADVIA Centaur[®] immunoassay (Siemens Healthcare Diagnostics Inc., Tarrytown, NY). PINP was measured using the Elecsys[®] PINP serum assay (Roche Diagnostics). CTX was measured using the Elecsys[®] β -Crosslaps serum assay (Roche Diagnostics).

Statistical analyses were performed using MedCalc[®]; 2.5th to 97.5th percentiles were calculated using nonparametric ranking. *t*-Test was used for comparison of means. A value of $P < 0.05$ was considered statistically significant. Regression analysis was used for correlation studies; 1 nmol/l of 25-OHD₃ is equivalent to 2.5 ng/ml.

Serum 25-OHD₃ concentrations ranged from 12.8 nmol/l (5.1 ng/ml) to 88 nmol/l (35.2 ng/ml). The 2.5th to the 97.5th percentile interval was 20.5 to 75.8 nmol/l (8.2–30.3 ng/ml). Table 1 shows the distribution of vitamin D status in the study cohort with the cut-point values based on the Institute of Medicine Report (2011) on Dietary Reference Intakes for Calcium and Vitamin D (8). Thirteen percent had increased risk of vitamin D deficiency (25-OHD₃ < 30 nmol/l) and 39% had increased risk of vitamin D inadequacy (25-OHD₃ < 40 nmol/l); 65% had 25-OHD₃ < 50 nmol/l. None had increased risk of vitamin D excess (25-OHD₃ > 125 nmol/l). There was no significant correlation between 25-OHD₃ concentrations and age. Vitamin D insufficiency (25-OHD₃ < 50 nmol/l) was more prevalent in Malays (89%) and Indians (82%) compared to Chinese (56%).

TABLE 1. Characteristics of Women with Vitamin D Deficiency, Insufficiency, and Sufficiency; Age is Presented as Median (95% Confidence Interval); PTH, PINP, and CTX are Presented as Mean (SD)

25-OHD ₃ (nmol/L)	<30	30–39.9	40–49.9	≥50
% (n)	13 (25)	26 (51)	27 (53)	35 (68)
Chinese % (n)	10 (14)	22 (29)	24 (32)	44 (59)
Malay % (n)	17 (6)	44 (16)	28 (10)	11 (4)
Indian % (n)	19 (5)	22 (6)	41 (11)	19 (5)
Age	38 (34–42)	40 (38–42)	43 (41–45)	37 (35–39)
PTH (pmol/l)	4.7 (1.51)	4.3 (1.46)	3.8 (1.34)	3.2 (1.22)
PINP (μg/l)	42.8 (13.83)	48.5 (19.29)	48.3 (17.11)	45.3 (24.18)
CTX (μg/l)	0.29 (0.130)	0.34 (0.143)	0.32 (0.160)	0.31 (0.159)

PTH ranged from 1.2 to 9.2 pmol/l. The 2.5th to the 97.5th percentile interval was 1.9–6.9 pmol/l. There was a positive correlation with age ($r = 0.173$; $P = 0.0001$). The average PTH concentration was significantly higher in Malays (4.5 ± 1.5 pmol/l) and Indians (4.4 ± 1.5 pmol/l) than in Chinese (3.6 ± 1.3 pmol/l). There was an inverse, linear correlation between PTH and 25-OHD₃ ($r = 0.383$; $P < 0.0001$), but no threshold of 25-OHD₃ concentrations at which PTH plateaued.

PINP concentrations ranged from 5.0 to 164.9 μg/l. The 2.5th to the 97.5th percentile interval was 19.5 to 92.0 μg/l. There was an inverse correlation with age ($r = 0.273$; $P < 0.0001$) but no significant difference between different ethnic groups. No significant correlation was found between PINP concentrations and 25-OHD₃ concentrations. Also, no significant correlation was found between PINP and PTH concentrations.

CTX ranged from 0.01 to 0.78 μg/l. The 2.5th to the 97.5th percentile interval was 0.08–0.66 μg/l. Similar to PINP, there was an inverse correlation between CTX concentrations and age ($r = 0.238$; $P = 0.01$) but no significant difference between different ethnic groups. The bone resorption marker CTX and the bone formation marker PINP exhibited good correlation ($r = 0.617$; $P < 0.0001$). However, no significant correlation was found between CTX concentrations and 25-OHD₃. Also, no significant correlation was found between CTX and PTH concentrations.

We found relatively high rates of vitamin D insufficiency (65%) in our cohort of healthy local women. This is consistent with the high prevalence of vitamin D insufficiency (30–80%) noted around the globe (9), and in the region (10, 11). However, a local study in older Chinese males and females found that 86% had 25-OHD concentrations ≥ 50 nmol/l (12). The accuracy of commercially available 25-OHD assays has been questioned (13), and the lack of comparability between different 25-OHD assays may also result in study differences.

Although our study cohort lived very close to the equator, where there is plenty of sunlight, they consist of hospital staff, who may spend long hours working indoors during the day. Besides inadequate sun exposure, vitamin D insufficiency may also be due to inadequate dietary intake. This finding of a high prevalence of vitamin D insufficiency in local healthy women raises the question of whether vitamin D supplementation should be advocated for healthy local women.

A local study comparing 25-OHD₃ concentrations between Chinese, Malays, and Indians in Singapore found lower 25-OHD₃ concentrations in Indians compared to Malays and Chinese (14). This study also found racial differences in vitamin D concentrations, with vitamin D insufficiency (25-OHD₃ < 50 nmol/l) more prevalent in Malays and Indians compared to Chinese. This may be because people with darker skin tone require longer sun exposure times to synthesize vitamin D in the skin (15). However, other factors such as diet, lifestyle and cultural habits may also play a role.

Malays and Indians not only had lower 25-OHD₃ concentrations compared to Chinese, they also had increased PTH concentrations, suggesting that 25-OHD₃ deficiency may affect parathyroid status. There was an inverse correlation between PTH and 25-OHD₃, but there was no threshold of 25-OHD₃ concentrations at which PTH plateaued, similar to other studies in the region (16). However, as there were very few women with 25-OHD₃ concentrations above 50 nmol/l (30 ng/ml), larger studies will be needed to confirm this relationship.

Both P1NP and CTX did not show significant differences in concentrations between the different ethnic groups. Other studies have shown differences between BMDs in different ethnic groups, with Chinese and Malays, but not Indians, exhibiting significant bone loss at the femoral neck (17). A local study demonstrated negative correlation between PTH and BMD at the hip and lumbar spine, but no correlation between 25-OHD and BMD (10). In contrast, another Asian study found a negative correlation between both CTX and P1NP and BMD (18). Therefore, it is still unclear whether the ethnic differences in 25-OHD₃ and PTH concentrations result in significant differences in bone metabolism.

Similar to other studies (19), there was no significant correlation between 25-OHD₃ concentrations and the bone turnover markers P1NP and CTX, suggesting that bone turnover markers are not useful biomarkers of vitamin D status (20).

This study has several limitations. First, in this cross-sectional study, the sample size is relatively small. Second, there are unequal numbers of the different ethnic groups. However, the proportion of different races is relatively similar to that of the population in Singapore. Third, the ages of the women spanned from 25 to 60

years, and included premenopausal and postmenopausal women. However, subgroup analyses did not detect significant differences in 25-OHD₃ between women below and above the age of 45. Fourth, it is not known whether any of the women were taking vitamin D supplements. However, none of the subjects had 25-OHD₃ concentrations above 100 nmol/l.

In conclusion, healthy women in Singapore have a high prevalence of vitamin D insufficiency. An inverse correlation between 25-OHD₃ and PTH was demonstrated. However, there was no correlation between the bone turnover markers P1NP and CTX with vitamin D or PTH.

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