Original Article

Effects of sun exposure on 25(OH) vitamin D concentration in urban and rural women in Malaysia

Musa Nurbazlin BBiomed[1], Winnie Siew Swee Chee PhD[2], Pendek Rokiah MRCP[1], Alexander Tong Boon Tan MRCP[1], Yee Yean Chew BSc[1], Abd Rahman Siti Nusaibah BSc[1], Siew Pheng Chan MRCP[1]

[1]*Department of Medicine, University of Malaya, Malaysia*
[2]*Department of Nutrition & Dietetics, International Medical University, Kuala Lumpur, Malaysia*

Ultraviolet B sunlight exposure is a primary source of vitamin D. There have been reports of low vitamin D status amongst the Malaysian population despite it being a tropical country. This study was conducted to determine the influence of sun exposure on 25(OH)D concentrations in urban and rural women in Malaysia and factors predicting 25(OH)D concentrations. Women aged above 45 years were recruited from urban (n=107) and rural areas (n=293). Subjects were interviewed regarding their outdoor activities and usual outdoor attire over the previous week. 25(OH)D concentrations were analyzed using the vitamin D3 (25-OH) electrochemiluminescence immunoassay. Median (Q1-Q3) age of the participants was 57 (53-61) years old. Median (Q1-Q3) 25(OH)D concentration of rural women was significantly higher [69.5 (59.0-79.1) nmol/L] compared to urban women [31.9 (26.1-45.5) nmol/L] (*p*<0.001). Rural women spent more time in the sun compared to urban women (7.83 (3.67-14.7) vs 2.92 (1.17-4.92) hours, *p*<0.001), although the fraction of body surface area (BSA) exposed to sunlight was significantly higher in the urban group [0.21 (0.21-0.43) vs 0.12 (0.07-0.17), *p*<0.001]. The calculated sun index (hours of sun exposure per week×fraction of BSA) was significantly higher in rural [0.89 (0.42-1.83)] compared to urban women [0.72 (0.26-1.28)], *p*=0.018. In the stepwise linear regression, rural dwelling increased the serum 25(OH)D by 31.74 nmol/L and 25(OH)D concentrations increased by 1.93 nmol/L for every unit increment in sun index. Urban women in Malaysia had significantly lower vitamin D status compared to rural women. Rural dwelling and sun index were key factors influencing vitamin D status in Malaysian women.

Key Words: 25(OH)D, sun exposure, body surface area, rural and urban women, Malaysia

INTRODUCTION

Vitamin D is a fat soluble steroid hormone which plays an important role in bone metabolism. Children and adults with vitamin D deficiency suffer from rickets and osteomalacia respectively. In addition, vitamin D insufficiency also increases the risk of bone loss and osteoporotic fractures in older people.[1] Previous studies have reported an association between vitamin D insufficiency and increased risk of chronic diseases such as colorectal cancer,[2] hypertension,[3] cardiovascular disease,[4] and diabetes mellitus.[5]

While vitamin D can be derived from dietary sources,[6] the primary source of vitamin D is from the activation of 7-dehydrocholesterol through the exposure of skin to ultraviolet B (UVB) radiation.[7,8] Hence, factors such as season, latitude, clothing, skin colour and time of day could influence vitamin D status.[7,8]

Pedersen reported that exposure to sunshine for 6 to 8 minutes, 2 to 3 times per week to the face, arms, hands and legs is more than sufficient to meet vitamin D requirements.[9] Given the hot climate all year round in tropical countries such as Malaysia, the population of these countries should have adequate concentrations of vitamin D. Several researchers have shown however, that there is a high prevalence of vitamin D insufficiency in tropical countries such as Thailand,[10] Vietnam,[11] India,[12] Hawaii,[13] Saudi Arabia,[14] and Bangladesh.[15]

Vitamin D insufficiency has also been reported in Malaysian children[16] and adults[17-19] despite perennial sunshine. These studies however,[7,16-19] were conducted mainly in urban settings and did not measure sun exposure and the potential influence of skin colour. The present study was conducted to determine factors influencing the 25(OH)D concentrations among urban and rural Malaysian women and the association to sun exposure.

MATERIALS AND METHODS
Study design and subjects
This was a cross-sectional study conducted from August 2010 to July 2011.

Corresponding Author: Ms Nurbazlin Musa, Department of Medicine, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia.
Tel: 603-79492622/603-79492260; Fax: 603-79556023
Email: nur_bazlin@yahoo.com; bazlin@siswa.um.edu.my

The study was conducted at University of Malaya Medical Centre (UMMC) (urban group) and Palong 1-11, Pusat Bandar Palong, Negeri Sembilan (rural group). From the population, a list of subjects who fulfilled the criteria were invited by phone (urban) or post (rural). A total of 107 urban and 203 rural women agreed to participate. Subjects were included if the following criteria were met: women aged above 45 years of age, community dwelling, independent in daily activities, bone mineral density (BMD) test with T-score >-2.5 (urban) and involved in the earlier ongoing research on a 10-year follow-up study of non-communicable diseases in a rural population in Malaysia for the year 2008-2010 enrollment (rural). Women who had a history of osteoporosis, cancer, chronic liver failure and chronic kidney failure were excluded from this study.

The study was approved by the Research Ethics Committee of UMMC [Medical Ethics Committee (MEC) reference no: 794.54 and 794.55]. Written informed consent was obtained from subjects.

Data collection

Demographic data such as date of birth, occupation and ethnicity were obtained from all subjects.

A fasting venous blood sample was collected for serum 25(OH) vitamin D and intact parathyroid hormone (i-PTH) analyses. The blood samples were kept at room temperature for 30 to 60 minutes to allow for clotting then spun at 3500 revolutions per minute (RPM) for 15 minutes. The serum was separated and stored at -80°C until vitamin D and i-PTH analysis. Vitamin D concentration was analyzed using electrochemiluminescence immunoassay (ECLIA) vitamin D3 (25-OH) method on the Cobas E-411 analyzer. The inter-assay coefficient of variation (CV) is 3.6% at 57.0 nmol/L (22.8 ng/ml) and 3.0% at 170.5 nmol/L (68.2 ng/ml). The intra-assay CV is 3.5% at 57.0 nmol/L (22.8 ng/ml) and 2.9% at 170.5 nmol/L (68.2 ng/ml). The measuring range for this kit is 10-250 nmol/L or 4-100 ng/mL.[20] Serum i-PTH was analyzed using ECLIA PTH on the Cobas E-411 analyzer. The inter-assay CV is 6.2% at 2.14 pmol/L (20.2 pg/ml) and 4.1% at 6.15 pmol/L (58.0 pg/ml). The intra-assay CV is 4.1% at 2.14 pmol/L (20.2 pg/ml) and 2.2% at 6.15 pmol/L (58.0 pg/ml). The measuring range for this kit is 0.127-530 pmol/L or 1.20-5000 pg/ml.[21] During the analytical run of vitamin D and i-PTH, two concentrations of control are run together with the samples to make sure the results are reliable.

The ECLIA vitamin D3 (25-OH) method was previously compared with other assay determining vitamin D concentrations which includes liquid chromatography–tandem mass spectrometry (LC-MS/MS), radioimmunoassay (RIA),[20,22] and high performance liquid chromatography (HPLC).[22] The comparison of the ECLIA method with each LC-MS/MS and RIA yielded a Passing/Bablok equation with y=1.008x + 0.045 (r=0.902; n=771) and y=0.899x + 5.146 (r=0.859; n=125) respectively.[20] The comparison of the ECLIA method with HPLC techniques yielded equation: Elecsys =1.077 × HPLC + 5.442 (S$_{y|x}$ = 13.9 nmol/L; n=67).[22]

Sun exposure was assessed using a questionnaire adapted from Heaney et al.[23] Subjects were asked about their outdoor activities over the previous week in terms of type of activity, duration (in minutes) and frequency (per week), usual outdoor attire and use of sunscreen and umbrellas. The "Rule of Nine" was used to estimate the fraction of body surface area (BSA) exposed to sunlight by the subject's attire during outdoor activity. The sun index, which is an index combining a measure of time outdoors during daylight and BSA usually exposed during that time, was calculated, as follows:

$$\text{Sun index} = (\text{hours of sun exposure per week}) \times (\text{fraction of BSA exposed to sunlight}).^{23}$$

Skin colour was measured using the Cosmetic Colour Ruler which has 16 points ranging from 1 (lightest) to 16 (darkest). This Ruler was applied to the darkest part of the body (taken as the back of the hand) and the lightest part of the body or natural skin colour (under the arm). The difference between the highest and lowest points of skin colour was calculated to determine delta skin colour.[13]

The subject's height was measured without shoes and head accessories using microtoise (Seca, Germany) to the nearest 0.1 cm. Weight and body fat percentages were measured with light clothing without socks and shoes using the Omron Body Composition Monitor with Scale Model HBF-362 Karada Scan which estimates the body fat percentage based on the bioelectrical impedance analysis.[24] Body mass index (BMI) was calculated as weight (kg) divided by height squared (m^2). Waist circumference was measured midway between the lowest rib and the top of the iliac crest using a measuring tape to the nearest 0.1 cm.

Dietary vitamin D intake was assessed using an FFQ over the past month. The FFQ was developed using methodology constructed by Chee et al.[25] The weights of food were adapted from the Nutrient Composition of Malaysian Foods.[26] Vitamin D content of raw foods was obtained from the United States Department of Agriculture (USDA) Standard Reference Database (Nutritionist Pro™ Software, First DataBank, Inc., San Bruno, CA). Vitamin D content in commercial products were obtained from product labels.[27] The total vitamin D intake (μg/day) was determined by multiplying the vitamin D content of the food, portion size and frequency of consumption.[25]

Statistical analysis

Data was analyzed using PASW SPSS, Version 18.0. Categorical data were described using count and percentages. Continuous data were checked for normal distribution (Kolmogorov-Smirnov). Data were recorded as median and quartiles (Q1-Q3) since the data were not normally distributed. Differences between population groups were evaluated using chi-Square and Mann-Whitney U tests. The correlation between two continuous variables was analyzed using Spearman correlation. A model using stepwise linear regressions was developed in order to determine the significance predictors of vitamin D. A p value of less than 0.05 was considered significant.

RESULTS

Participants' characteristics

A total of 400 women (293 urban and 107 rural) participated in this study. Median (Q1-Q3) age of the participants was 57 (53-61) years old. The majority of urban

subjects were Chinese (52.3%), followed by Indians (28.0%) and Malays (19.6%). Rural subjects were mainly Malays (84.0%), followed by Indians (12.3%) and Chinese (3.8%).

The majority of the rural participants were housewives (82.6%) whilst 11.9% worked outdoors as rubber tappers or farmers. Most of the urban women were retired (45.8%). 29.0% were housewives and 25.2% worked indoors. None of the urban women worked outdoors (Table 1).

Rural women were significantly shorter in height compared to their urban counterparts, however there was no significant difference in their body weight, leading to a higher median BMI. In terms of body fat percentages, there was no significant difference between the two groups of women, although rural women had higher waist circumference than urban women.

Rural women had significantly darker skin colour compared to urban subjects. However, delta skin colours were not significantly different. The rural women had darker skin colour regardless of their ethnicity (data not shown).

Vitamin D and i-PTH status
Rural women had significantly higher median concentra-

tions of 25(OH)D and i-PTH compared to urban women (Table 1). In Table 2, 25(OH)D concentrations were classified based on the cut-off points given by Institute of Medicine (IOM), whereby 25(OH)D<30 nmol/L (<12 ng/mL) as vitamin D deficient, 30-<50 nmol/L (12-<20 ng/mL) as vitamin D insufficient and ≥50 nmol/L (≥20 ng/mL) as vitamin D sufficient.[28,29] (To convert 1 nmol/L to 1 ng/mL is to divide by 2.5).[19] Using these cut-off points, the majority of urban women (43.9%) were vitamin D deficient, whilst the majority of rural women (88.1%) were vitamin D sufficient.

According to the manufacturer, the normal range for serum i-PTH should be 1.6-6.9 pmol/L.[21] Using these cut-off, 91.3% of women have normal range of serum i-PTH, while 0.5% of women had values below and 8.0% had values above the normal range of serum i-PTH. Only 6.3% of those women who had vitamin D deficiency had elevated PTH value (data not shown). Comparing the different classification of vitamin D status, there were no significant different in serum i-PTH concentrations between deficient vs insufficient (4.08 vs 3.90 pmol/L, p=0.665), deficient vs sufficient (4.08 vs 3.96 pmol/L, p=0.827) and insufficient vs sufficient (3.90 vs 3.96 pmol/L, p=0.727) concentrations of vitamin D in overall population. Only rural women who had vitamin D insuf-

Table 1. Demographic, physical and biochemical parameters

	Urban (n=107)	Rural (n=293)	p	Overall (n=400)
Age, years[†]	61 (58-65)	56 (52-59)	<0.001	57 (53-61)
Ethnicity[‡]			<0.001	
Malay	21 (19.6)	246 (84.0)		267 (66.8)
Chinese	56 (52.3)	11 (3.8)		67 (16.8)
Indian	30 (28.0)	36 (12.3)		66 (16.5)
Occupation[‡]			<0.001	
Working indoors	27 (25.2)	14 (4.8)		41 (10.3)
Working outdoors	0 (0)	35 (11.9)		35 (8.8)
Retired	49 (45.8)	2 (0.7)		51 (12.8)
Housewife	31 (29.0)	242 (82.6)		273 (68.3)
Height (cm)[†]	154 (151-157)	151 (147-156)	<0.001	152 (148-156)
Weight (kg)[†]	61.9 (54.9-68.3)	62.7 (57.2-70.8)	0.125	62.6 (56.5-70.2)
BMI (kg/m²)[†]	26.0 (23.1-29.0)	27.9 (25.2-30.5)	<0.001	27.2 (24.9-30.1)
Waist circumference(cm)[†]	90.0 (82.0-96.4)	94.0 (88.0-102)	<0.001	93.0 (87.0-100)
Body fat percentage (%)[†]	38.7 (36.0-41.2)	37.8 (35.5-40.4)	0.095	38.1 (35.7-40.6)
Darkest skin colour score[†]	9 (7-12)	12 (11-14)	<0.001	12 (10-13)
Lightest skin colour score[†]	4 (2-8)	6 (4-9)	<0.001	5 (4-9)
Delta skin colour[†, §]	5 (4-6)	6 (3-7)	0.138	5 (3-7)
25(OH)D, nmol/L[†, ¶]	31.9 (26.1-45.5)	69.5 (59.0-79.1)	<0.001	64.5 (45.6-75.3)
PTH, pmol/L[†, ††]	3.59 (2.78-4.79)	4.13 (3.14-5.51)	0.011	3.96 (3.08-5.26)

[†] Data as median (Q1-Q3)
[‡] Data as n (%)
[§] Delta skin colour/the changes of the skin colour = Darkest skin colour – Lightest skin colour.[13]
[¶] 25(OH)D conversion factor: nmol/L × 0.40 = ng/ml.[20]
[††] PTH conversion factor: pmol/L × 9.43 = pg/ml.[21]

Table 2. Classification of Vitamin D status and corresponding PTH levels

25 (OH)D[†]	Urban (n=107)		Rural (n=293)		Overall (n=400)	
	n (%)	PTH level[‡]	n (%)	PTH level[‡]	n (%)	PTH level[‡]
Deficient (<30 nmol/L)	47 (43.9)	4.07 (3.15-4.89)	1 (0.3)	4.33 (4.33-4.33)	48 (12.0)	4.08 (3.16-4.89)
Insufficient (30-<50 nmol/L)	40 (37.4)	3.53 (2.57-4.48)	34 (11.6)	4.92 (3.55-6.68)[§, ¶]	74 (18.5)	3.90 (3.09-5.39)
Sufficient (≥50 nmol/L)	20 (18.7)	3.39 (2.40-4.81)	258 (88.1)	3.96 (3.10-5.27)	278 (69.5)	3.96 (3.04-5.26)

[†] Vitamin D cut-off points using IOM.[28, 29]
[‡] Data as median (Q1-Q3), PTH unit: pmol/L
[§] Significantly different from sufficient, p<0.0167 (Mann-Whitney U tests with Bonferroni correction)
[¶] Significantly different from urban

Table 3. Linear and non-linear equations for the relationship between serum 25(OH)D and PTH in urban and rural groups

	Urban	Rural
Linear	PTH = 4.316 – 0.011 x 25(OH)D; R^2=0.022, p=0.125	PTH = 5.752 – 0.019 x 25(OH)D; R^2=0.031, p=0.002
Logarithmic	PTH = 5.640 – 0.496 x ln[25(OH)D]; R^2=0.026, p=0.099	PTH = 9.858 – 1.287 x ln[25(OH)D]; R^2=0.031, p=0.022
Cubic	PTH = 6.027 – 0.139 x 25(OH)D + 0.003 x $[25(OH)D]^2$ – 1.571 x 10^{-5} x $[25(OH)D]^3$; R^2=0.050, p=0.148	PTH = 3.226 + 0.103 x 25(OH)D – 0.002 x $[25(OH)D]^2$ + 8.290 x 10^{-6} x $[25(OH)D]^3$; R^2=0.038, p=0.011
Power	PTH = 6.331 x $[25(OH)D]^{-0.156}$; R^2=0.039, p=0.040	PTH = 13.541 x $[25(OH)D]^{-0.284}$; R^2=0.032, p=0.022
Exponential	PTH = 4.221 x $e^{-0.004}$; R^2=0.041, p=0.037	PTH = 5.485 x $e^{-0.004}$; R^2=0.032, p=0.022

Table 4. Sun exposure and sun index

	Urban (n=107)	Rural (n=293)	p
Sun exposure per week (h)	2.92 (1.17-4.92)	7.83 (3.67-14.71)	<0.001
Fraction of BSA exposed to sunlight	0.21 (0.21-0.43)	0.12 (0.07-0.17)	<0.001
Sun Index[†]	0.72 (0.26-1.28)	0.89 (0.42-1.83)	0.018

Data as median (Q1-Q3)
[†] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight.[23]

ficiency showed significantly higher concentration of serum i-PTH compared to rural women who had vitamin D sufficiency (4.92 vs 3.96 pmol/l, p=0.003). At the insufficient vitamin D concentration, there was a significant difference in serum i-PTH between urban and rural groups (3.53 vs 4.92 pmol/L, p=0.006) (Table 2).

There was no correlation between serum i-PTH and 25(OH)D concentrations (Spearman's rho=0.048, p=0.342). The linear and non-linear models were performed to further characterized the relationship between serum 25(OH)D and i-PTH in urban and rural groups. The equations were shown in Table 3. Some of the models fitted well but the predictive value of the models was low (only explained by 2.2-5.0%).

Sun exposure
Malaysia is a tropical country which has abundant sunshine, consistent temperature, high humidity and plentiful rainfall. It is very rare to have a day with completely no sunshine in our country. Overall, Malaysia receives about 6 hours of sunshine per day.[30]

Rural women spent significantly more time under the sun (7.83 hours/week) compared to urban women (2.92 hours/week). When body surface exposed to sunlight is taken into account, rural women still had a higher sun index score compared to urban women. The urban women had a higher percentage of body parts exposed to sunlight, but the duration of exposure to sunlight was short (Table 4).

In terms of sunscreen use, 6.5% urban subjects reported frequent application of sunscreen when exposed to the sun, 18.7% reported occasional sunscreen application and 74.8% reported no use of sunscreen. Among the rural subjects, 5.5% routinely applied sunscreen, 5.1% occasional sunscreen use and 89.4% reported no sunscreen use.

Amongst the Malay women, 6.7% reported frequent, 7.1% occasional and 86.1% no application of sunscreen respectively. Amongst the Chinese, 4.5% reported frequent, 14.9% occasional and 80.6% reported no use of sunscreen; while similar results were 3.0% frequent, 9.1% occasional and 87.9% no use of sunscreen in Indian women (data not shown).

Table 5 displays the correlation between duration of sun exposure per week, fraction of BSA exposed to the sun and calculated sun index to the 25(OH)D concentrations. Hours of sun exposure per week and Sun Index were positively correlated with 25(OH)D concentrations. Conversely, fraction of BSA exposed to the sun was negatively correlated with 25(OH)D concentrations.

Vitamin D intake
Rural women had significantly higher median (Q1-Q3) intake of vitamin D compared to urban women [5.23 (3.31-8.45) vs 4.61 (2.66-7.41) μg/day]. The recommended vitamin D intake for Malaysian women aged 30-50 years, 51-65 years and >65 years is 5, 10 and 15 μg/day, respectively.[31] Only 7.5% (n=8) of urban women and 19.1% (n=56) of rural women met the recommended nutrient intake (RNI)[31] for vitamin D. The median (Q1-Q3) total vitamin D intake for both urban and rural women who were vitamin D deficient, insufficient and sufficient were 3.55 (2.57-7.43), 4.74 (3.09-7.32) and 5.27 (3.14-8.56) μg/day, respectively. Serum 25(OH) vitamin D concentration did not correlate with vitamin D intake (Spearman's rho=0.084, p=0.095).

Factors predicting vitamin D status
The factors known to affect vitamin D concentrations were determined using stepwise linear regression with serum 25(OH)D concentration as dependent variables and hours of sun exposure per week, fraction of BSA exposed to the sun, sun index, dietary vitamin D intake and dwelling (i.e., urban vs rural) as independent variables. The R^2 value of the model was 0.415 (data not shown). Rural dwelling and sun index were major determinants of vitamin D concentrations. Residing in rural area increased

Table 5. Bivariate correlation between 25 (OH) vitamin D3 and sun exposure

	25 (OH) vitamin D3	
	Spearman's rho	p
Hours of sun exposure per week	0.342**	<0.001
Fraction of BSA exposed to sunlight	-0.264**	<0.001
Sun index[†]	0.180**	<0.001

Data as Spearman's rho
[†] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight.[23]
** Correlation is significant at the 0.01 level (2-tailed).

Table 6. Factors predicting serum 25(OH)D concentrations assessed by stepwise linear regression

Independent Variables	25(OH)D concentrations, nmol/L		
	Unstandardized coefficient[†]	Standardized coefficient[†]	p
Area[‡]	31.7 (27.9, 35.6)[¶]	0.62	<0.001
Sun index[§]	1.93 (0.64, 3.22)	0.11	0.003

[†] β coefficient
[‡] Area, 1 for rural and 0 for urban area
[§] Sun index = hours of sun exposure per week x fraction of BSA exposed to sunlight.[23]
[¶] 95% CI in parentheses (all such values)

serum 25(OH)D by 31.7 nmol/L. In addition, 25(OH)D concentrations increased by 1.93 nmol/L for every unit increment in sun index (Table 6). Hours of sun exposure per week (p=0.360) and fraction of BSA exposed to the sun (p=0.486) individually were not significant predictors of vitamin D (data not shown). But, the combination of them, which is Sun Index (hours of sun exposure per week × fraction of BSA exposed to sunlight)[23] were significant predictors of vitamin D. Dietary vitamin D intake (p=0.451) also was not significant determinant of vitamin D (data not shown).

Higher body fat is expected to reduce availability of circulating vitamin D.[17] In our study, body fat percentage (p=0.061) was not significantly associated with vitamin D concentrations. This may be due to the high prevalence of overweight and obesity (BMI ≥23 kg/m^2)[32] (86.3%), with a median BMI 27.2 (24.9-30.1) kg/m^2 in our study population (data not shown).

DISCUSSION
A preliminary analysis conducted by Chan *et al* reported a significant difference in mean 25(OH) vitamin D concentrations between urban and rural subjects, whereby rural subjects had higher 25(OH) vitamin D concentrations compared to urban subjects [70.7±18.3 vs 45.7±15.5 nmol/L (or 28.3±7.3 vs 18.3±6.2 ng/mL)].[33] The present study support the previous findings[33,34] whereby rural women in this cohort also had significantly higher median concentration of vitamin D compared to urban women.

The cut-off point of vitamin D status remains controversial.[17] Two concentrations of 25(OH)D is normally used to defined vitamin D insufficiency which are <75 or <50 nmol/L (<30 or <20 ng/mL).[35] For example, a report by IOM in 2010 suggested that vitamin D concentrations of ≥50 nmol/L (or ≥20 ng/mL) contributed to the requirements of at least 97.5% of the population.[28] Recently,

International Osteoporosis Foundation and DSM Nutritional Products developed the global map to illustrate the vitamin D status and they defined vitamin D >75 as optimal, 50-74 as suboptimal, 25-49 as insufficient, and <25 nmol/L as deficient, respectively.[36] In addition, the US Endocrine Society Clinical Practice Guideline determined Vitamin D deficiency as 25(OH)D below 50 nmol/L (20 ng/mL), and insufficiency as 52.5-72.5 nmol/L (21-29 ng/mL).[37] However, vitamin D concentration >75 nmol/L (>30 ng/mL) are not consistently associated with increased benefits.[28] Overall, most researchers define vitamin D insufficiency as 25(OH)D concentrations below 50 nmol/L.[38] Using the cut-off points defined by IOM,[28,29] we found that higher percentages of urban women (43.9%) had vitamin D deficiency compared to rural women (0.3%). In contrast, 18.7% of urban women and 88.1% of rural women were vitamin D sufficient. However, our finding revealed a high prevalence (81.3%) of vitamin D <50 nmol/L in urban women compared to previous studies[17] regardless of vitamin D assay used. Foong *et al.* also reported a high prevalence (87%) of urban females in Kuala Lumpur with vitamin D concentration <50 nmol/L.[19]

Using the same assay as ours, 40% of Vietnamese women aged between 30-60 years, and 56% of Vietnamese women aged >60 years had vitamin D insufficiency [25(OH)D<75 nmol/L or <30 ng/ml].[11] The prevalence of their finding is similar with our findings that 47.1% and 52.9% of our urban women aged ≤60 and >60 years had vitamin D <75 nmol/L, respectively (data not shown).

Green *et al* reported the vitamin D status of nonpregnant women aged 18-40 years residing in Kuala Lumpur. Their results showed that higher percentages of Indian (68%) and Malay (74%) women had vitamin D insufficiency [25(OH)D<50 nmol/L] compared to Chinese (38%) women.[7] Similarly, in our study, a higher percentage of urban Malay (95.2%) and Indian (83.3%) women had vitamin D insufficiency compared to Chinese (75.0%) women. This could be due to the darker skin colour of Malay and Indian women compared to Chinese women resulting in higher melanin contents which can inhibit the cutaneous synthesis of vitamin D.[17]

While an inverse correlation between serum vitamin D and PTH concentration has been reported elsewhere,[11,17] but our study showed that there was no correlation between serum intact PTH and 25(OH) vitamin D concentrations. This result agrees with Elsammak *et al.* who reported no association between PTH concentrations with serum vitamin D concentrations in male and female subjects.[39] As a result, PTH concentrations should not be used clinically as an indicator of vitamin D deficiency.[13] Therefore, regardless of calcium and PTH results, serum 25(OH) vitamin D should be measured if vitamin D insufficiency/deficiency is predicted.[39]

The threshold concentrations of serum vitamin D to indicated vitamin D deficiency can be predicted based on the relationship between serum 25(OH)D and PTH.[11] For example, based on the relationship between serum 25(OH)D and i-PTH that fitted well in the exponential non-linear regression model, Islam *et al* concluded that the plateau for the i-PTH concentrations was at 21 ng/L and vitamin D concentrations >38 nmol/L maintain low

concentrations of i-PTH.[15] On the other hand, Ho-Pham *et al* reported that there was no cut-off point of vitamin D concentrations at which PTH concentrations plateau since none of the spline regression model fitted well compared to the simple linear regression model.[11] In urban and rural women in our study, the non-linear curve between serum PTH and 25(OH)D concentrations showed poor relationship and did not plateau. This might be because the majority of subjects who had vitamin D deficiency or insufficiency had normal PTH values. In addition, PTH molecules are easily broken down in blood after blood taking because it is unstable,[40] resulting in difficulties to get the actual results. The differences in vitamin D status between urban and rural groups could be explained by skin colour or sun exposure. Rural women generally had darker skin than their urban counterparts, regardless of ethnicity and had higher sun exposure due to the nature of their work which is more outdoors than indoors.[17] Darkening of exposed skin has been shown to be associated with women who spent more time outdoors.[13] Tsiaras *et al.* reported that individuals with higher melanin content or darker skin pigmentation require longer sun exposure compared to individuals with lighter skin pigmentation to produce an equivalent amount of vitamin D3.[41] There were positive correlations between darkest skin colour and delta skin colour with 25(OH) vitamin D concentration. However, no correlation was found between lightest skin colour and vitamin D concentration (data not shown).

Rural dwelling and sun index were the significant predictors of vitamin D in the stepwise multiple linear regression model. Rural women spent more time under the sun, but had less body surface area exposed to the sun. However, the sun index was significantly higher in rural women. This indicated that although rural women were more covered-up (due to religion and cultural practices), the duration spent under the sun was greater, resulting in higher vitamin D concentrations. Most of rural women were housewives, who reported that they spent a lot of time performing outdoor activities such as hanging clothes, sweeping the porch and gardening. Rural women who worked were more like to work outdoors compared to urban women who mostly worked indoors.

Harinarayan *et al* reported a low prevalence of 25(OH) vitamin D deficiency in rural subjects in Andhra Pradesh, India compared to urban subjects. Rural subjects who work as agricultural laborers were more exposed to the sun in terms of style of clothing and the duration of exposure about 8 hours per day.[42] On the other hand, Bangladeshi females who worked inside a garment factory for 14-16 hours every day had low serum vitamin D (36·7 nmol/L).[15]

It has been reported that being exposed to the sun 2-3 times a week for about 10-15 minutes (ie, 20 to 45 minutes per week) results in sufficient vitamin D production by the skin.[15] However, urban women in this study still had low vitamin D concentrations despite reporting sun exposure of 2.92 hours/week with 21% of BSA exposed to the sun. This result might be due to subjects overestimating their sun exposure, obstruction of sunshine by tall buildings, air pollution,[15] and the texture and colour of clothing.[43]

As stated above, the percentage of urban women who apply sunscreen when exposed to the sun was higher compared to rural women. The application of sunscreen with Sun Protection Factor 8 suppresses the cutaneous synthesis of vitamin D.[44] The photoconversion of 7-deydrocholesterol to previtamin D3 is disrupted by 5% para-aminobenzoic acid (PABA) sunscreen to the skin.[41] For instance, the mean concentration of serum vitamin D of the groups who did not use sunscreen when going outdoors was half point higher than those group who applying PABA sunscreen to the exposed parts of the body before being outside.[45] Vitamin D intake did not appear to be a significant contributor to 25(OH) vitamin D status in majority of these women. Most urban and rural women did not meet the RNI for vitamin D intake. This was similar to another study, where only 12% of 60 urban adults in Malaysia met the RNI for vitamin D.[27] There was no correlation between dietary Vitamin D intake and serum 25(OH) vitamin D concentration, similar to what was reported by Puri *et al.*[46]

The concentrations of 25(OH)D can be determined using various methods such as RIA, HPLC, and LC-MS (liquid chromatography–mass spectrometry). These methods can measure both 25(OH)D2 and 25(OH)D3.[22] However, each method has advantages and disadvantages. For example, although HPLC is consider as the gold standard method, however, it requires large volume of samples and a well-trained technician.[47] Moreover, although the precision of LC-MS/MS techniques has been approved, it tends to overestimated the reading of serum 25(OH)D concentrations than its actual value.[48] This may be due to the assay interference which unable to distinguish between 25(OH)D3 and its inactive isomer 3-epi-25(OH)D3.[47] Consequently, lower prevalence of vitamin D insufficiency was reported by this technique.[48] The limitation in our assay is its inability to measure 25(OH)D2. As a result, it may be underestimate the vitamin D status. However, the assay used in the study was in good overall agreement as measured by LC-MS/MS and RIA.[22]

25(OH)D which is lipophilic in nature cause matrix effect which reduce the capability of the binding agent, antibody, or binding protein to attach with 25(OH)D in the sample or standard.[49-50] The matrix effect such as lipid or anything present in the samples was not a big factor when using HPLC or LC-MS method to analyze vitamin D.[49] However, vitamin D assay using RIA or Liaison was affected by matrix effects.[50] The insert kits of our assay stated that interferences may be caused by visible sign of hemolyzed sample. On the other hand, the assay is unaffected by icterus (bilirubin <205 μmol/L), lipemia (intralipid <400 mg/dL) and biotin (<82 nmol/L).

Another limitation includes the sun exposure was based on self-report and patient recalls of sun exposure over the previous week. The estimation of vitamin D intake also has limited accuracy due to the lack of a database on Malaysian foods.

In conclusion, 25(OH) vitamin D concentration was positively associated with the number of hours of sun exposure per week and the Sun Index, and negatively correlated with fraction of BSA exposed to the sun. Urban women in Malaysia had significantly lower sunlight

exposure and vitamin D status compared to rural women. Our study has shown that rural dwelling and sun index were the major factors influencing vitamin D status in Malaysian women.

ACKNOWLEDGEMENTS

AUTHOR DISCLOSURES

REFERENCES

1. Schoenmakers I, Goldberg GR, Prentice A. Abundant sunshine and vitamin D deficiency. Br J Nutr. 2008;99:1171-3. doi: 10.1017/S0007114508898662

2. Feskanich D, Ma J, Fuchs CS, Kirkner GJ, Hankinson SE, Hollis BW, Giovannucci EL. Plasma vitamin D metabolites and risk of colorectal cancer in women. Cancer Epidem Biomar. 2004;13:1502-8.

3. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willett WC, Curhan, GC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. Hypertension. 2007;49:1063-9. doi: 10.1161/HYPERTENSIONAHA.107.087288

4. Wang TJ, Pencina MJ, Booth SL, Jacques PF, Ingelsson E, Lanier K, Benjamin EJ, D'Agostino RB, Wolf M, Vasan RS. Vitamin D deficiency and risk of cardiovascular disease. Circulation. 2008;117:503-11. doi: 10.1161/CIRCULATIONAHA.107.706127

5. Catharina M, Paul K, Satu M, Harri R, Maarit AL, Jukka M, Reunanen A. Serum 25-hydroxyvitamin D concentration and subsequent risk of type 2 diabetes. Diabetes Care. 2007; 30:2569-70. doi: 10.2337/dc07-0292

6. Burgaz A, Åkesson A, Oster A, Michaelsson K, Wolk A. Associations of diet, supplement use, and ultraviolet B radiation exposure with vitamin D status in Swedish women during winter. Am J Clin Nutr. 2007;86:1399-404.

7. Green TJ, Skeaff CM, Rockell JEP, Venn BJ, Lambert A, Todd J et al. Vitamin D status and its association with parathyroid hormone concentrations in women of child-bearing age living in Jakarta and Kuala Lumpur. Eur J Clin Nutr. 2008;62:373-8. doi: 10.1038/sj.ejcn.1602696

8. Woo J, Lam CWK, Leung J, Lau WY, Lau E, Ling X et al. Very high rates of vitamin D insufficiency in women of child-bearing age living in Beijing and Hong Kong. Brit J Nutr. 2008;99:1330-4. doi: 10.1017/S0007114507844382

9. Pedersen JI. Recommended intake, dietary intake and upper tolerable levels of vitamin D. Solar Radiation and Human Health. 2008;229-37.

10. Chailurkit L, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC Public Health. 2011;11: 853. doi: 10.1186/1471-2458-11-853

11. Ho-Pham LT, Nguyen ND, Lai TQ, Eisman JA, Nguyen TV. Vitamin D status and parathyroid hormone in a urban population in Vietnam. Osteoporos Int. 2011;22:241-8. doi: 10. 1007/s00198-010-1207-4

12. Zargar AH, Ahmad S, Masoodi SR, Wani AI, Bashir MI, Laway BA, Shah ZA. Vitamin D status in apparently healthy adults in Kashmir Valley of Indian subcontinent. Postgrad Med J. 2007;83:713-6. doi: 10.1136/pgmj.2007. 059113

13. Binkley N, Novotny R, Krueger D, Kawahara T, Daida YG, Lensmeyer G, Hollis BW, Drezneret MK. Low vitamin D status despite abundant sun exposure. J Clin Endocrin Metab. 2007;92:2130-5. doi: 10.1210/jc.2006-2250

14. Sadat-Ali M, Abdulmohsen A, Haifa A, Fatma A, Amein A. Vitamin D levels in healthy men in eastern Saudi Arabia. Ann Saudi Med. 2009;29:378-82. doi: 10.4103/0256-4947. 55168

15. Islam MZ, Shamim AA, Kemi V, Nevanlinna A, Akhtaruzzaman M, Laaksonen M, Jehan AH, Jahan K, Khan HU, Lamberg-Allardt C. Vitamin D deficiency and low bone status in adult female garment factory workers in Bangladesh. Br J Nutr. 2008;99:1322-9. doi: 10.1017/S000711450889 4445

16. Khor GL, Chee WSS, Shariff ZM, Poh BK, Arumugam M, Rahman JA, Theobald HE. High prevalence of vitamin D insufficiency and its association with BMI-for-age among primary school children in Kuala Lumpur, Malaysia. BMC Public Health. 2011;11:95. doi: 10.1186/1471-2458-11-95

17. Suriah AR, Chee WSS, Zaitun Y, Chan SP. Vitamin D status among postmenopausal Malaysian women. Asia Pac J Clin Nutr. 2004;13:255-60.

18. Foong MM. Vitamin D status and its associated factors of free living Malay adults in a tropical country, Malaysia. J Photochem Photobiol B. 2011;104:444–8. doi: 10.1016/j. jphotobiol.2011.05.002

19. Foong MM, Awang B. High prevalence of vitamin D insufficiency and its association with obesity and metabolic syndrome among Malay adults in Kuala Lumpur, Malaysia. BMC Public Health. 2011;11:735. doi: 10.1186/1471-2458-11-735

20. Vitamin D3 (25-OH) [package insert]. Mannheim, Germany: Roche Diagnostics GmbH; 2010.

21. Parathyroid hormone (parathormone, parathyrin) – PTH, intact [package insert]. Mannheim, Germany: Roche Diagnostics GmbH; 2010.

22. Leino A, Turpeinen U, Koskinen P. Automated measurement of 25-OH vitamin D3 on the Roche Modular E170 Analyzer. Clin Chem. 2008;54:2059-62. doi: 10.1373/clinchem.2008.111732

23. Barger-Lux JM, Heaney RP. Effects of above average summer sun exposure on serum 25-hydroxyvitamin D and calcium absorption. J Clin Endocr Metab. 2002;87:4952-6. doi: 10.1210/jc.2002-020636

24. Omron Instruction Manual Body Composition Monitor with Scale Model HBF-362 Karada Scan [package insert]. Kyoto, Japan: Omron Healthcare Co., LTD; 2009.

25. Chee WSS, Suriah AR, ZaitunY, Chan SP, Yap SL, Chan YM. Dietary calcium intake in postmenopausal Malaysian women: comparison between the food frequency questionnaire and three-day food records. Asia Pacific J Clin Nutr. 2002;11:142-6. doi: 10.1046/j.1440-6047.2002.00276.x

26. Tee ES, Ismail MN, Nasir MA, Idris K. Nutrient composition of Malaysian foods (komposisi zat dalam makanan Malaysia). 4th ed. Kuala Lumpur: Institute for Medical Research; 1997.

27. Lee HPP. Sunlight exposure and vitamin D intake amongst older adults in Klang Valley, Malaysia [unpublished degree's thesis]. Malaysia: International Medical University; 2011.

28. Ross AC, Manson JE, Abrams SA, Aloia JF, Brannon PM, Clinton SK et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: what clinicians need to know. J Clin Endocrinol Metab. 2011;96:53-8. doi: 10.1210/jc.2010-2704

29. Institute of Medicine. Dietary reference intakes for calcium and vitamin D. Washington, DC: The National Academies Press; 2011.

30. Official Portal Malaysian Meteorological Department [Internet]. Malaysia: Ministry of Science, Technology and Innovation (MOSTI); c2012 [cited 2013 Mar 30]. General climate of Malaysia; [about 3 screens]. Available from: http://www.met.gov.my/index.php?option=com_content&task=view&id=75&Itemid=1089&limit=1&limitstart=0

31. Technical Working Group on Nutritional Guidelines. A report of the technical working group on nutritional guidelines: recommended nutrient intakes for Malaysia. Malaysia: National Coordinating Committee on Food and Nutrition (NCCFN) Ministry of Health Malaysia; 2005.

32. WHO expert consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet. 2004; 363:157-63. doi: 10.1016/S0140-6736(03)15268-3

33. Chan SP, Chew YY, Vijay A, Lim SS, Tan ATB, Hazizi AS, Zaitun Y, Joseph A, Manivasagar M, Rokiah P. Vitamin D inadequacy in Malaysia: preliminary analysis of a rural vs urban population. Proceedings of the Endocrine Society 91st Annual Meeting; 2009 Jun 10-13; Washington, DC.

34. Tan ATB, Shalena N, Joseph A, Chew YY, Vijay A, Lim SS, Hazizi AS, Rokiah P, Chan SP. Vitamin D in postmenopausal women: preliminary analysis of an urban vs rural cohort in Malaysia. Proceedings of the 14th Asia Oceania Congress of Endocrinology; 2010 Dec 2-5; Kuala Lumpur, Malaysia. Kuala Lumpur: Malaysian Endocrine and Metabolic Society; 2010. p. 38.

35. Dawson-Hughes B, Mithal A, Bonjour JP, Boonen S, Burckhardt, P, Fuleihan GEH, Josse RG, Lips P, Morales-Torres J, Yoshimura N. IOF position statement: vitamin D recommendations for older adults. Osteoporos Int 2010;21:1151-54. doi: 10.1007/s00198-010-1285-3.

36. DSM Nutritional Products. Global vitamin D map launched – DSM calls for urgent action to address major public health issue. Kaiseraugst, Switzerland: Press Release; 2012.

37. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP, Murad MH, Weaver CM. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab. 2011;96: 1911-30. doi: 10.1210/jc.2011-0385

38. Zittermann A, Gummert JF. Sun, vitamin D, and cardiovascular disease. J Photochem Photobiol B. 2010;101: 124-9. doi: 10.1016/j.jphotobiol.2010.01.006

39. Elsammak MY, Al-Wossaibi AA, Al-Howeish A Alsaeed J. High prevalence of vitamin D deficiency in the sunny Eastern region of Saudi Arabia: a hospital-based study. EMHJ. 2011;17:317-22.

40. Sturgeon CM, Sprague SM, Metcalfe W. Variation in parathyroid hormone immunoassay results-a critical governance issue in the management of chronic kidney disease. Nephrol Dial Transplant. 2011;26:3440-5. doi: 10.1093/ndt/gfr614

41. Tsiaras WG, Weinstock MA. Factors influencing vitamin D status. Acta Derm-Venereol. 2011;91:115-24.

42. Harinarayan CV, Ramalakshmi T, Prasad UV, Sudhakar D. Vitamin D status in Andhra Pradesh: A population based study. Indian J Med Res. 2008;127:211-18.

43. Mallah EM, Hamad MF, ElManaseer MA, Qinna NA, Idkaidek NM, Arafat TA, Matalka KZ. Plasma concentrations of 25-hydroxyvitamin D among Jordanians: effect of biological and habitual factors on vitamin D status. BMC Clinical Pathology. 2011;11:8. doi: 10.1186/1472-6890-11-8

44. McCarty CA. Sunlight exposure assessment: can we accurately assess vitamin D exposure from sunlight questionnaires? Am J Clin Nutr. 2008;87:1097S-101S.

45. Norval M, Wulf HC. Does chronic sunscreen use reduce vitamin D production to insufficient levels? Br J Dermatol. 2009;161:732-6. doi: 10.1111/j.1365-2133.2009.09332.x

46. Puri S, Marwaha RK, Agarwal N, Tandon N, Agarwal R, Grewal K, Reddy DHK, Singh S. Vitamin D status of apparently healthy schoolgirls from two different socioeconomic strata in Delhi: relation to nutrition and lifestyle. Br J Nutr. 2008;99:876-82. doi: 10.1017/S0007114507831758

47. Hollis BW. Measuring 25-hydroxyvitamin D in a clinical environment: challenges and needs. Am J Clin Nutr. 2008; 88:507S-10S.

48. Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, Petruschke RA, Chen E, Papp AED. Prevalence of vitamin D Inadequacy among postmenopausal North American women receiving osteoporosis therapy. J Clin Endocrinol Metab. 2005;90:3215-24. doi: 10.1210/jc.2004-2364

49. Hollis BW. Assessment of circulating 25(OH)D and 1,25(OH)2D: emergence as clinically important diagnostic tools. Nutr Rev. 2007;65:S87-S90. doi: 10.1301/nr.2007.aug. S87-S90

50. Barake M, Daher RT, Salti I, Cortas NK, Al-Shaar L, Habib RH, Fuleihan GE. 25-hydroxyvitamin D assay variations and impact on clinical decision making. J Clin Endocrinol Metab. 2012;97:835-43. doi: 10.1210/jc.2011-2584

Original Article

Effects of sun exposure on 25(OH) vitamin D concentration in urban and rural women in Malaysia

Musa Nurbazlin BBiomed[1], Winnie Siew Swee Chee PhD[2], Pendek Rokiah MRCP[1], Alexander Tong Boon Tan MRCP[1], Yee Yean Chew BSc[1], Abd Rahman Siti Nusaibah BSc[1], Siew Pheng Chan MRCP[1]

[1]*Department of Medicine, University of Malaya, Malaysia*
[2]*Dept of Nutrition & Dietetics, International Medical University, Kuala Lumpur, Malaysia*

陽光曝曬對於馬來西亞城鄉婦女血液中 25-羥基維生素 D 濃度之影響

維生素 D 的主要來源,是由陽光中紫外線 B 照射皮膚所合成。儘管馬來西亞屬於熱帶國家,仍有研究指出部分馬來西亞人口處於低維生素 D 的狀態。本研究目的為評估陽光曝曬對於居住在都市與鄉村的馬來西亞婦女,血液 25-羥基維生素 D 的影響,及其濃度的預測因子。共招募 400 位 45 歲以上女性,其中 107 位居住於都市,293 位居住於鄉村。面訪參與者以詢問過去一個禮拜的戶外活動情形及通常的戶外衣著。利用電化學發光免疫分析法,測定血清 25-羥基維生素 D 的濃度。參與者的年齡中位數為 57 歲(53-61 歲)。鄉村女性維生素 D 的中位數值顯著高於都市女性(69.5 nmol/L 比上 31.9 nmol/L;$p<0.001$)。儘管都市女性暴露在陽光下的面積較高(0.21 比上 0.12;$p<0.001$),但陽光曝曬的時間顯著低於鄉村女性(2.92 比上 7.83 小時;$p<0.001$)。比起都市女性,鄉村女性有顯著較高的陽光曝曬指數(每週陽光曝曬時數 x 曝曬體表面積)。逐步線性迴歸顯示居住於鄉村者,血清 25-羥基維生素 D 濃度高出 31.7 nmol/L;每增加一單位陽光曝曬指數,血清 25-羥基維生素 D 濃度升高 1.93 nmol/L。馬來西亞都市婦女的維生素 D 狀態,顯著較差於鄉村女性。居住於鄉村及陽光曝曬指數,是影響馬來西亞婦女維生素 D 狀態的主要因素。

關鍵字:25-羥基維生素 D、陽光曝曬、體表面積、鄉村及都市女性、馬來西亞