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CALCIUM AND VITAMIN D STATUS OF KELANTANESE MALAY WOMEN FROM LOW INCOME FAMILY: A POPULATION-BASED STUDY

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Abstract: *Objectives:* The objective of this study is to determine the calcium and vitamin D status of Malay women from low income family in Kelantan. *Design:* The study design is a cross-sectional study. *Setting:* Kelantan, Malaysia. *Participants:* Calcium and vitamin D status were determined in 150 (51 pre and 99 postmenopausal) healthy Kelantanese Malay women. *Measurements:* Calcium intake was determined by diet recall and food frequency questionnaire, while vitamin D based on serum levels of 25-hydroxyvitamin D (25 (OH) D). *Results:* The data showed that the mean calcium intake was 492.9±316.51 mg/day where 86.3% of the pre- and 91.9% of the postmenopausal subjects have calcium intakes lower than the recommended daily intake for Malaysian women (800 mg/day and 1000mg/day respectively). The intake of calcium was affected by calorie intake and education level (P<0.05). Whilst for vitamin D, 16.4% of the subjects were classified under Hypovitaminosis D, 82.9% insufficient vitamin D level and another 0.7% is under deficiency category. None of the subjects have sufficient circulating level of 25 (OH) vitamin D. Vitamin D intake showed significant correlation with BMI (P<0.05). *Conclusion:* These findings, which showed that majority of the subjects, have low calcium and vitamin D status is a concern as it will lead to public health problems, especially osteoporosis. Strategy needs to be developed in combating this problem in the lower income populations. Educating the public to raise consciousness towards healthy eating and maintaining a healthy body mass index, and calcium with vitamin D supplementation in the selected high risk groups might be a cost-effective preventative measure.

Key words: Calcium, vitamin D, 25-hydroxyvitamin D (25 (OH) D), premenopausal women, postmenopausal women.

Introduction

Good nutritional status is important in maintaining humans' well-being, growth and development, and resistance towards illnesses (1). The rate of mortality in Malaysia is largely contributed by the unhealthy heating habits and inactive lifestyles (Malaysian Dietary Guidelines 2010). One of the factors that related to these contributors is socioeconomic status. It is widely reported that the lower income community has poorer health status compared to the higher income community. Poor calcium and vitamin D status is detrimental towards skeletal health. Calcium and vitamin D deficiency leads to the reduction of mineralization and growth of bone, reducing its structural stiffness and strength (2, 3).

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Apart from their importance in maintaining skeletal health, calcium and vitamin D also play important role in many cellular activities (4, 5). Every cell in human body depends on the calcium in extracellular fluid (ECF) in order to function properly. Calcium ion involves in metabolic function and also acts as one of the linkages between the extracellular coordination functions such as nerve signals, endocrine controls and cytokine action (4). Due to their crucial roles in our body, calcium ion concentration is strictly regulated in the ECF in about 4.8mg/100ml (1.20 mmol/L) (6). Calcium is obtained through diet, and for Malaysian, the suggested requirement is at 800 mg/day for premenopausal and 1000 mg/day for postmenopausal women (7, 8). Vitamin D is involved in many physiological processes as well as to maintain muscle strength (5, 9). Vitamin D is obtained either from diet or through the synthesis under the skin after exposure to sunlight.. Vitamin D, in form of 1,25-(OH)2D3 also suppresses the formation of parathyroid hormone (PTH) which promotes bone loss (3, 5, 10). Being the major active circulating metabolites, 25(OH)D is regarded as the indicator of vitamin D status (11, 12). Vitamin D deficiency happens when the circulating

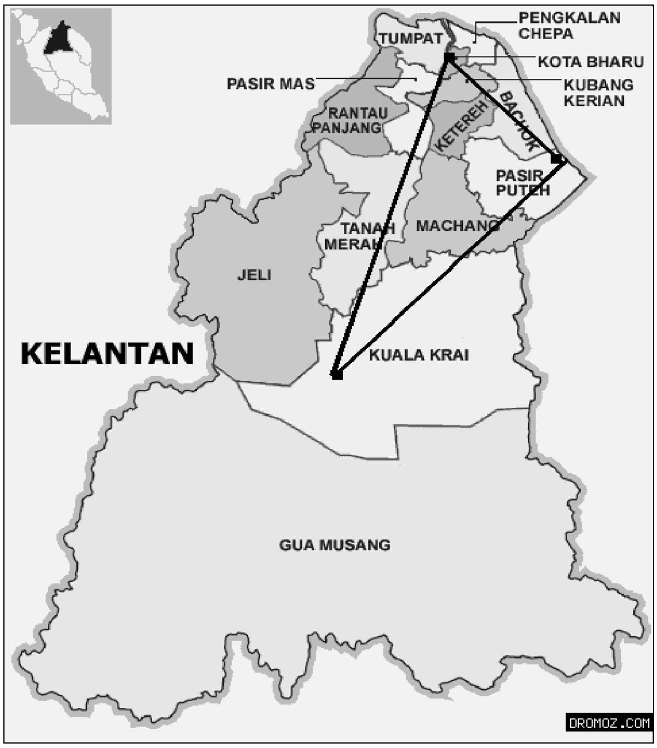
25(OH) vitamin D is less than 10 ng/mL. With vitamin D deficiency, there will be higher risks of rickets, osteomalcia, calcium malabsorptions, severe hyperparathyroidism, and impaired immune and cardiac function. Vitamin D insufficiency (10-19.9 ng/mL) leads to the reduction of bone mineral density, impaired muscle function, decrease in the rate of intestinal calcium absorption, and increased of PTH level. Hypovitaminosis D (20-39.9 ng/mL) leads to a slight elevation of PTH. Vitamin D status is adequate at 40 ng/mL, but intoxication occurred when it exceeds 100 ng/mL. 1 ng/mL equals to 2.5 nmol/L (13). An extensive review on vitamin D status in various countries showed that there is a strong relationship between the status and life-style (14).

It is widely accepted that nutrition intake is different for different population. There are many factors which influenced the calcium and vitamin D status in a population, including race, ethnicity, and geographic location (4, 12, 15, 16). Aging will also influence calcium and vitamin D status. It has been acknowledged that ovarian hormones affect calcium and vitamin D metabolism. When a women experience pre menopausal stage, there will be alteration in calcium and vitamin D homeostasis (17). In Malaysia, the average age of menopause is 47.96 years, and as the life-span is increasing, people will spend longer time in pre and postmenopausal stage (18). It is important that they have sufficient level of calcium and vitamin D in this stage. In many industrialized countries, it is shown that people with lower socioeconomic status leads to poor health, which might be due to the limitation in financial resources (12, 19). Hence, it is relevant to study the nutrient intake in this bottom billionth population. In this study, we aimed to identify the calcium and vitamin D status of Malay women from low income family in Kelantan, and also to determine the factors influencing calcium and vitamin D status in this population.

Methods

150 subjects were recruited in three different districts to represent the population in Kelantan, Malaysia (Figure 1). 51 healthy premenopausal women (aged 45 and above with regular menstrual cycle for the past six months) and 99 healthy postmenopausal women were recruited. Menopausal state was indicated by the absence of menstrual cycle for 12 months. Exclusion criteria were pregnancy, breast feeding, taking drugs known to affect bone metabolism (glucocorticoids, HRT, calcium and vitamin D supplement), had evidence of medical or surgical condition known to affect bone loss (eg. hysterectomy, bilateral oophorectomy), having critical illnesses or chronic (osteoporosis, rheumatoid arthritis, cancer, diabetes). All subjects were recruited from lower income family, based on Pendapatan Garis Kemiskinan (PGK) 2009 by Economic Planning Unit, Prime Minister's Department Malaysia (total income RM770 in urban and RM740 in rural area).

Figure 1
Sampling Population



Blood samples were taken from subjects between 800 am to 1100 am after an overnight fasting. Serum 25(OH)-vitamin D3 was determined using 25(OH)-Vitamin D direct ELISA (Immundiagnostik AG, Stubenwald-Allee, Bensheim, Germany) kit. Results were expressed in ng/mL. Both the intra-assay and inter-assay precision (CV) were 7% (n=20). For this paper, vitamin D deficiency is defined as serum 25(OH)D lower than 10 ng/mL, insufficiency serum 25-OH vitamin D level is 10 to 19.9 ng/mL, and hypovitaminosis D level is 20 to 39.9 ng/mL (13).

Daily calcium intake and calorie intake were obtained from the Food Frequency Questionnaire (FFQ) and 24-hour diet recall. Subjects were interviewed individually by trained dietitians. The FFQ consists of a total 30 food items that are rich in calcium content. The list was divided into 5 different food groups (milk and milk products, cereal and nuts, fruits and vegetables, seafood, and egg). The subjects were asked about the frequency of intake and also the number of servings for each food item. The amount of calcium intake was calculated based on Nutrient Composition of Malaysian Foods (1997), and for commercialized food, the calcium content was calculated based on the nutrition information label on the packaging. The calcium intake for each subject was averaged from both FFQ and Diet recall. The sufficiency of calcium intake was based on the recommended daily intake for Malaysian women, which are 800 mg/day for premenopausal group, and 1000mg/day for postmenopausal group (7, 8).

Socio-economic, lifestyle habit, and clinical data were obtained by interview with trained panels. Lifestyle questionnaire were derived from the iDecide Physical Activity Score (with permission from Abbott Laboratory). Body weight was measured in light clothing and without shoes to the nearest 0.1 kg using weighing scale, and standing height was measured using bodymeter to the nearest 0.1 cm. Body mass index (BMI) was then calculated for each subject.

Data analysis was performed using the Statistical Package for Social Sciences version 18 (SPSS, IBM Corporation, Chicago, IL, USA). Statistical significance was set at the 5% level. Results were expressed in mean ± standard deviation. Log transformation was done for skewed data. One-way analyses of variance (ANOVA), t-test, Pearson's correlation, Tukey's test, and linear and multiple linear regressions were used whenever appropriate to determine the correlation and significant differences.

Results

The characteristics of subjects were shown in Table 1. The majority of the subjects were married women, with some education background. None went to tertiary level of education. The average income of the subjects was RM 451.26±200.78 (USD 147.45±65.60) per month. There were significant differences between postmenopausal and premenopausal groups in age, marital status, education level, and monthly income ($P<0.05$). 51% of the subjects were housewives. Among those who work, 45% were farmers, mostly rubber tappers. The mean calorie intake for all subjects was 6041 ± 2109 kJ per day.

There was no significant difference between the anthropometric characteristics of the two groups (Table 2). Majority of the subjects were non-smokers. Majority of the pre-menopause subjects had modest activity score (76.5%), which includes cycling, gardening, and walking.

The calcium intake obtained from the average of FFQ and diet recall was not normally distributed. Following log transformation, the data formed a normal distribution. The means were taken from the anti-log of the data. In our study, the women in this population have low calcium intake. The average intakes were 524.10±285.80 mg/day for the pre menopausal group and 476.76±331.46 mg/day for the post menopausal group, which is significantly lower than the recommended daily intakes (Table 2). Although the postmenopausal subjects seemed to have lower daily calcium intake compared to the premenopausal subjects, it was not significantly different. 86.3% of the premenopausal and 91.9% of the postmenopausal subjects have calcium intakes lower than the recommended daily intake for Malaysian women.

Table 1
Demographic Characteristics of Subjects (n = 150)

Variables	Premenopause (n =51)		Postmenopause (n = 99)	
	Mean	SD	Mean	SD
Age (year) *	49.1	2.76	59.22	6.16
Marital Status **				
Married	84.3%		65.7%	
Divorced	9.8%		6.1%	
Widowed	5.9%		28.3%	
Number of children	7	3	6	3
Education level **				
No education	21.6%		34.3%	
Primary School	29.4%		46.5%	
Secondary School	49.0%		18.2%	
Religious School	0%		1.0%	
Work				
Working	51.0%		46.5%	
Housewife	43.1%		45.5%	
Pensioner	5.9%		7.1%	
Pensioner and working	.0%		1.0%	
Monthly income (RM)*	510.2	262.36	421.96	208.94
Source of income				
Pension	.0%		5.1%	
Salary	35.3%		36.4%	
Children	25.5%		40.4%	
Charity	2.0%		.0%	
Others	37.3%		18.2%	

Mean (SD): * mean difference is significant at $P<0.05$ by one-way ANOVA, Tukey's test. ** mean difference is significant at $P<0.05$ by Pearson Chi-Square test

Table 2
Anthropometric characteristics, lifestyle habits, and calcium intake of subjects (n=150)

Variables	Premenopause (n =51)		Postmenopause (n = 99)	
	Mean	SD	Mean	SD
Height (cm)	151.0	6.59	149.7	4.76
Weight (kg)	59.6	11.32	57.8	12.36
BMI (kg/m^2)	26.1	4.11	25.7	5.19
BMI category				
Underweight[1]	2.0%		5.2%	
Normal[2]	40.0%		44.3%	
Overweight[3]	42.0%		29.9%	
Obese[4]	16.0%		20.6%	
Smoking status				
Smoker	7.8%		8.1%	
Ex-smoker	.0%		6.1%	
Non-smoker	92.2%		85.9%	
Physical activity				
Sedentary	7.8%	30.3%		
Modest activity	76.5%	48.5%		
Active lifestyle	15.7%		20.2%	
Very active lifestyle	.0%		1.0%	
Mean calcium intake (mg/day)				
Subjects' intake	524.1 (285.80)		476.8 (331.46)	
Recommended intake	800 (7)		1000 (8)	

Mean (SD): 1. defined as BMI < 18.5 kg/m^2; 2. defined as BMI 18.5–24.9; 3. defined as BMI 25–29.9; 4. defined as BMI >30

The serum vitamin D data was normally distributed. The mean serum vitamin D level was 17.36 ± 2.80 ng/mL. 16.4% of the subjects were classified under Hypovitaminosis D, 82.9% insufficient vitamin D level and another 0.7% is under deficiency category. None of the subjects have sufficient circulating level of 25 (OH) vitamin D (Table 3). The mean circulating vitamin D level is not significantly different for all sampling population.

Table 3
Vitamin D status of Subjects (n=150)

Vitamin D Status (ng/mL)	Premenopausal (n=51)		Post menopause (n=99)		All Subjects (n=150)	
	Mean	SD	Mean	SD	Mean	SD
Mean	16.96	2.72	16.73	2.79	17.36	2.80
Category (%)						
Deficiency (<10)	2.2		-		0.7	
Insufficient (10-19.9)	86.7		81.1		82.9	
Hypovitaminosis D (20-39.9)	11.1		18.9		16.4	

Simultaneous multiple regression was conducted to investigate the best predictors of calcium intake and vitamin D in this population. Dummy variables were created for the categorical independent variables. The combination of variables to predict calcium intake was statistically significant ($P< 0.01$) with the adjusted R2 value of .384 indicating 38% of the variance was explained by the model. The significant predictors are calorie intake ($P< 0.01$), education level and serum vitamin D level ($P< 0.05$) (Table 4). Low calorie intake leads to low calcium intake. Subjects with secondary school education showed significantly higher intake of calcium compared to those with no education (589.45 ± 313.88 mg/day vs. 436.52 ± 367.24 mg/day) ($P<0.05$). The difference is not significant from those with primary school education (461.30 ± 263.18 mg/day).

Table 4
Simultaneous multiple regression analysis summary in predicting factors influencing calcium intake (n=150)

Variable	B	SEB	β
Age	-.005	.457	-.065
Number of children	-.002	.015	-.010
Amount of monthly income	.000	.000	.054
BMI	-.011	.008	-.085
Calorie intake	.001	.000	.602**
Education level			
Primary education	.165	.116	.136
Secondary education	.424	.128	.322*
Serum vitamin D level	-.299	.136	-.181*

Note: R^2 = .384, $p< 0.01$; B = Unstandardized coefficients; SEB = Standard error of B
β = Standardized coefficients (correlation coefficients); ** $p<0.01$, * $p<0.05$

The combination of variables to predict circulating vitamin D level was statistically significant ($p< 0.05$) with the adjusted R2 value of .099 indicating 10% of the variance was explained by the model (Table 5). The only significant predictor is body mass index (BMI), which is negatively correlated with serum vitamin D level ($p< 0.05$, β -.257).

Table 5
Simultaneous multiple regression analysis summary in predicting factors influencing circulating vitamin D level (n=150)

Variable	B	SEB	β
Age	.019	.079	.019
Amount of monthly income	-.002	.002	-.082
BMI	-.361	.001	-.257*
Education level			
Primary education	-.380	1.033	-.028
Secondary education	-1.121	1.137	-.075
Calorie Intake	.001	.000	-.098
Physical activity			
Modest activity	1.182	1.095	.086
Active activity	.684	1.333	.040

Note: R^2 = .099, $p< 0.05$; B = Unstandardized coefficients; SEB = Standard error of B
β = Standardized coefficients (correlation coefficients); * $p<0.05$

Discussion

The calcium intake for this population is notably low compared to Malaysian RNI, with a mean of 493 mg/day compared to the 1000 mg/day that is recommended. This intake is similar to the intake of Chinese postmenopausal Malaysian women studied by Chee et. al (2002), which shows an intake of was 447+/-168 mg/day and 499+/-211 mg/day from dietary records and FFQ respectively. However, this is higher than the intake found by Suriah et. al (1996) on elderly population in peninsular Malaysia, which is extremely low, from a mean of 276.97 mg to 302.51 mg/day.

The high percentage of subjects with low calcium intake can be attributed to both poor choices of food and low calorie intake in the population. The subjects consumed low amount of dairy and soy products which were two of the main sources of calcium. Only a small number of the subjects consumed milk on regular basis, while most of them preferably consumed plain tea or coffee. The calcium intake is affected by education level, where subjects with secondary education showed higher calcium intake compared to those without proper education. This shows that subjects with better education is more likely consumed adequate nutrition. This is supported by other studies which show that subjects that come from educated family were more likely to eat sufficient dairy products, fruits and vegetables (19, 21).

Besides low calcium intake, this population also suffered from low circulating serum 25(OH)D. The vitamin D insufficiency in this population was not unexpected as extensive review on various countries had yielded similar results, even within countries with sufficient sunlight (14, 22). In this study, 50% of the subjects were housewives, and it was shown that Malay tend to stay indoors and avoid extreme heat of mid-day sun (16). Even though individuals only need to be exposed to sunlight for about 15 minutes during mid-day to obtained adequate vitamin D level, the clothing habits of Malay women which only exposed the UV radiation to face and hands, as well as dark pigmentation, reduced the synthesis of vitamin D (14, 16).

The low vitamin D status might also be due to the age of our subjects. Aging decreases the functional capacity of the skin to synthesis vitamin D (23). It has been documented that upon similar radiation, the synthesis of vitamin D3 was four times slower than young adult (14). Aging also leads to estrogen deficiency, and as estrogen is involved in converting 25(OH)D to its actives form and regulates calcium metabolism, it contributes to low calcium and vitamin D status in this population (17, 24). This decreased in vitamin D synthesis and metabolism lead to the dependency on dietary source in order to maintain adequate level of serum vitamin D.

We also observed an inverse correlations between BMI and serum 25(OH)D. Several studies had indicated that vitamin D deficiency was associated with higher body fat (25 – 28). As we failed to measure the body fat of the subjects, this association could not be made. Wortsman et al. studied the bioavailability of vitamin D in obese people and found that obesity did not reduce the efficiency of skin to synthesis vitamin D, however, as vitamin D is fat soluble, it is not readily release into circulation, but being deposited into adipose tissue.

In this study, smokers made up of only 10% of the population, hence, any correlations between smoking and vitamin D cannot be determined. However, many studies had related smoking with the reduction of serum vitamin D levels, hence, indirectly affecting the bone health (29-32). The mechanism on how smoking affecting the circulation level of 25(OH) is yet to be determine. One of the factors might be due to the caffeine content in cigarettes, where Rapuri et al. suggested that caffeine affects vitamin D receptors. Hansdottir et al. (2010) showed that cigarette smokes reduced the conversion of vitamin D into its active form. However, this study was only focus on vitamin D metabolism in lungs.

The low calcium intake and vitamin D level in this population might lead to various health complications, especially fracture and osteoporosis. Studies had shown that vitamin D deficiency leads to increased risk of rickets, decrease bone mineral content and increase risk of bone fracture (34). Lower calcium intake will reduce bone calcium content, making it more fragile and prone to fracture (35). As education is associated with good nutrient intake, it was suggested that the public being educated about the importance of these nutrients whenever they consult a physician (36). It is widely accepted that whenever there is a deficient, supplementation is desirable (8, 36). A number of randomized control trials had shown a positive increase in serum 25(OH)D and bone density after using calcium and vitamin D supplementation (35, 37- 39). Vitamin D supplementation on its own will have little effect on bone health status and is most effective when coupled with calcium (34). Although vitamin D synthesis in skin reduced due to aging, but the absorption was shown to be adequate (14). Hence, to prevent any health complications, calcium and supplementation might be a good way, apart from education the population on healthy eating and maintaining healthy BMI.

Our study has several limitations. We did not measure the body fat which might influence the serum vitamin D level. Apart from that, we also did not measure the time exposed to sunlight. Additional research might be done to measure the bone density of the women in this population.

Conclusion

In summary, there was a high level of calcium and vitamin D inadequacy among pre and postmenopausal Kelantanese Malay women. In this study, it was shown that education influenced the calcium and vitamin D status; hence, public education is important to raise consciousness towards healthy eating and maintaining a healthy body mass index. Besides that, calcium and vitamin D supplementation might be a cost effective measure in combating this deficiency. These strategies hopefully will help this populace to have higher quality of life in their late years.

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