RESEARCH ARTICLE

Prevalence and predictors of hypovitaminosis D among the elderly in subtropical region

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Abstract

The prevalence of low vitamin D status in the elderly population of subtropical area and the potential risk/protective factors have not been addressed. This cross-sectional question-naire-based study, which collected demographic/anthropometric data and information on diet habit and sun exposure, recruited 170 subjects with mean age 70.9 \pm 5.6 in rural areas of southern Taiwan. Serum 25-OH vitamin D, calcium, and intact parathyroid hormone were also measured. Using cut-off level of 30 ng/mL, subjects were divided into low (n = 95) and normal (n = 75) serum vitamin D groups. The results demonstrated a low vitamin D status in 30.6% of men and 57.7% of women. Dietary vitamin D intake was another factor associated with vitamin D status (p = 0.02). Logistic regression identified inadequate intake of vitamin D-rich food as the only risk factor for low vitamin D status in men (OR = 4.55, p = 0.01), whereas inadequate sun exposure was the only predictable risk with dose-response relationship in women (low vs. high sun exposure, OR = 6.84, p = 0.018; moderate vs. high sun exposure, OR = 6.67, p = 0.005). In conclusion, low vitamin D status was common in the elderly of subtropical rural areas. Low sun exposure and inadequate dietary vitamin D consumption were associated with a low vitamin D status in females and males, respectively.

Introduction

Vitamin D, which is one essential nutrient for intestinal calcium absorption to facilitate mineralization of bone [1], not only helps in maintaining muscle strength and function [2], but has also been reported to reinforce immunity [3, 4] as well as improve outcomes of cardiovascular diseases [5–7]. At the other end of the spectrum, vitamin D deficiency has been shown to be associated with rickets, osteomalacia, osteoporosis, risk of fracture, and cancers [1, 8–10]. It has also been reported to be associated with colorectal, lung, prostate, breast and ovarian cancer [11]. Consistently, vitamin D supplementation has been reported to reduce all-cause



Citation: Huang C-H, Huang Y-TA, Lai Y-C, Sun C-K (2017) Prevalence and predictors of hypovitaminosis D among the elderly in subtropical region. PLoS ONE 12(7): e0181063. https://doi.org/10.1371/journal.pone.0181063

Editor: Susanne Kaser, Medical University Innsbruck, AUSTRIA

Received: November 24, 2016

Accepted: June 25, 2017

Published: July 31, 2017

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Data Availability Statement: Data are available from doi:10.6084/m9.figshare.5162119.

Funding: The authors received no specific funding for this work.

Competing interests: The authors have declared that no competing interests exist.

cancer mortality [12]. Moreover, vitamin D supplementation seems to lower the risk of acute respiratory infection and asthma exacerbation [13, 14].

Vitamin D can be produced by the skin through exposure to ultraviolet-B (UVB) or obtained from the diet. UVB converts 7-dehydrocholesterol to pre-vitamin D3 in the skin, followed by conversion to vitamin D3. On the other hand, vitamin D2 (ergocalciferol) is acquired exclusively from diet. Vitamin D (i.e., vitamin D2 and D3) is metabolized in the liver to 25-hydroxyvitamin D (25-(OH)D), which is often used as a biomarker to determine vitamin D status [15]. Although vitamin D production has been found to be adequate as long as there is sunlight exposure of arms and legs for 5 to 30 minutes twice weekly [16, 17], low vitamin D status remains a public health problem worldwide [18].

Although there is no definite consensus so far, vitamin D deficiency and insufficiency are usually defined as 25-hydroxyvitamin D < 20 ng/mL and 20–30 ng/mL, respectively [1, 2, 19– 21]. According to large-scale cross-sectional studies in North America, one-third of all subjects was demonstrated to have 25-(OH)D level below 20 ng/mL, two-thirds of those below 30 ng/ mL [22, 23]. In other words, low vitamin D status is a common condition in western populations. On the other hand, in addition to the dearth of study on the prevalence of low vitamin D status in Asia [18, 24, 25], there is no investigation addressing the issue in subtropical countries. Moreover, the target population in previous studies focused mainly on adolescents and young adults rather than the elderly whose population has been on the increase worldwide. Indeed, aging is known to be associated with decreased 7-dehydrocholesterol concentration in the skin, resulting in reduction of vitamin D3 production [17, 26, 27]. Interestingly, although previous studies carried out across different countries in Southern Asia revealed a high prevalence of low vitamin D status, no particular attention has been paid to the elderly population [28, 29]. Although risk factors for a low vitamin D status (i.e., age, gender, race, body mass index, latitude, season, nutritional intake, sun exposure, and physical activity) have been reported for the general population [23, 29-31], risk and protective factors contributing to the vitamin D status in the elderly have not be addressed. The purposes of the study, therefore, is to determine the prevalence of low vitamin D status in the elderly population living in a subtropical area and to explore the potential risk or protective factors.

Subjects and methods

Study population

Healthy volunteers aged 65 or older were randomly recruited from a tertiary referral hospital in a rural area in Southern Taiwan from February to August 2015. Community dwellers older than 65 years with written informed consent were eligible for the present study, whereas those with active cancer in previous 5 years, end-stage renal disease, liver cirrhosis, psychiatric disease, or disorders known to affect bone mineral metabolism including hyperthyroidism, hyperparathyroidism, osteomalacia, Crohn's disease, cystic fibrosis, or Paget's disease were excluded.

Study design and parameters

This is a prospective cross-sectional study based on information acquired through questionnaires and blood samples. After signing the informed consent, each subject was required to complete a questionnaire for collecting information on age, gender, body height, body weight, past history, medical and drug history, status of vitamin D supplement, status of sun exposure, and consumption of vitamin D-rich food through a food diary. According to the total diet scores, the participants were classified into those with high vitamin D intake and those with relatively low intake. Blood samples were obtained from all subjects for the quantification of 25-hydroxyvitamin D (25-(OH)D), intact parathyroid hormone (iPTH), and serum calcium at the central laboratory of the institute. The whole study protocol was reviewed and approved by the Institutional Review Board of E-Da Hospital (EMRP-102-045). Informed consents were obtained from all subjects.

Questionnaire design

The instrument used for assessing the factors affecting an individual's vitamin D status was a questionnaire containing four major items, including (1) basic demographic, anthropometric, and clinical information including medical history, drug history (2) consumption of vitamin D supplements, including type and dosage (3) extent of sun exposure and habits of sunscreen use (4) food frequency questionnaire (FFQ) regarding the consumption of vitamin D-rich food. To estimate the amount of vitamin D from food, a food frequency questionnaire (FFQ) was developed by an experienced dietician (S1 and S2 Tables). The vitamin D contents of the commonly available vitamin D-rich food items included in the questionnaire are summarized in supporting information S3 Table [1, 32]. In each item, consumption was recorded by frequency per week, which was transformed into scores (i.e., never = 0 point, 1–2 times/week = 1 point, more than 2 times/week = 2 points). The sum of total scores was used to estimate the intake during recent 3 months. Physical activity was measured by using Taiwanese version of the International Physical Activity Questionnaire, which has been shown to be a valid and reliable tool [33].

Blood tests

Five milliliters of blood was obtained from each subject between 10 a.m. and 11 a.m. in the morning. All subjects were refrained from calcium-containing food and beverage including calcium supplement tablets, and milk as well as caffeine- or alcohol-containing beverage for at least 12 hours before collection of blood samples. Serum levels of 25-hydroxyvitamin D (25-(OH)D), intact parathyroid hormone (iPTH) were determined at the central laboratory of the institute with standard methods using chemiluminescent immunoassay (Abbott ARCHITECT i2000, Illinois, U.S.A.). Serum calcium level was determined by o-cresolphthalein complexone (OCPC) method (Toshiba C, Tokyo, Japan). All analyses were completed within 12 hours of blood sample harvesting.

Definitions

Degree of sun exposure was divided into three categories according to the duration between 8 a.m. and 5 p.m.: Low (<1 hour/week), medium (1–2 hours/week), and high (>2 hours/week). Sun screen use was defined as the use of any sun screen products within 3 months prior to the study regardless of the frequency. Vitamin D supplements were defined as all oral supplements containing vitamin D other than food. A diet scoring system was developed based on the frequency of consumption of vitamin D-rich food products including fish, sardine, herring, salmon, oysters, eggs, fortified cereals, fortified soymilk, and fortified milk. The testing subjects were categorized into low (score 0–5) and high (score 6–10) diet scorers. The four seasons were defined as: Spring (March—May), summer (June—August), fall (September—November), and winter (December—February). Three levels of Physical activity, which were proposed by the scoring protocol in guidelines for data processing and analysis of the Taiwan version of International Physical Activity Questionnaire (IPAQ), were used to label participants as low, moderate, and high groups[33]. The definition of vitamin D deficiency and insufficiency are usually defined as 25-hydroxyvitamin D < 20ng/mL and 20-30ng/mL, respectively

[1]. The testing subjects in our study were divided into low and normal vitamin D groups if their serum 25-OH vitamin D levels were < 30 ng/mL and ≥ 30 ng/mL, respectively.

Statistical analysis

All statistical analyses were carried out using the IBM SPSS for Windows software program version 22.0 (Armonk, NY: IBM Corp, USA). A p-value <0.05 was considered significant. Demographic and anthropometric data are expressed as mean ±standardized deviation (SD). Pearson correlation was adopted to explore the relationship between serum 25(OH)D and iPTH level. Chi-square test was used for determination of significance of difference between categorical variables. Multiple logistic regression analysis was performed to study the association between each presumed risk factor and vitamin D status, including age, gender, sun exposure, and vitamin D intake.

Results

Comparison of baseline characteristics between participants with low and normal serum vitamin D levels

Totally 170 volunteers were recruited, including 85 men and 85 women with a mean age of 70.9 \pm 5.6 years (range, 65–85) and a mean BMI of 24.0 \pm 3.2 kg/m2 (range, 15.1–34.2) which was around the upper limit of normal (Table 1). Most participants had medium sun exposure (48.2%) without the habit of sunscreen use (67.1%) (Fig 1). In addition, most of them consumed adequate vitamin D through normal diets (67.1%) without the habit of taking vitamin D supplements (77.1%). According to the serum vitamin D levels, there were 75 (44.1%) and 95 (55.9%) testing subjects belonging to the low and normal vitamin D group, respectively. A low vitamin D status was demonstrated in 30.6% men and 57.7% of women. Gender was a significant indicator of vitamin D status. The majority of participants with low vitamin D status were women (65.3%) (p < 0.001). Dietary intake of vitamin D was another factor significantly associated with vitamin D status (p = 0.02). Age, BMI, sun exposure, use of sun screen, vitamin D supplement, season of blood withdrawal, or physical activity were not associated with vitamin D status.

Correlations among serum vitamin D, calcium, and iPTH concentrations

The participants showed a mean serum 25-OH vitamin D level of 30.9 ± 8.6 ng/mL, ranging from 13 to 79 ng/mL. On the other hand, their mean serum iPTH level was 53.0 ± 22.3 pg/mL,

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	Total (N = 170)	Low vitamin D group (N = 95) (25-OH vitamin D<30 ng/mL)	Normal vitamin D group (N = 75) (25-OH vitamin D≧30 ng/mL)	*P value
Age, years (mean ± SD)	70.9 ± 5.6	70.4 ± 5.7	71.2 ± 5.5	0.36
Gender				
Male, N (%)	85 (50%)	26 (34.7%)	59 (62.1%)	<0.001
Female, N (%)	85 (50%)	49 (65.3%)	36 (37.9%)	
Height, cm (mean ± SD)	159.5 ± 11.7	158.8 ± 15.4	160.0 ± 8.0	0.51
Weight, kg (mean ± SD)	60.9 ± 9.6	60.0 ± 9.7	61.7 ± 9.5	0.25
BMI, kg/m ² (mean ± SD)	24.0 ± 3.2	23.9 ± 3.4	24.1 ± 3.1	0.81

*Significance of difference determined by Chi-square test

https://doi.org/10.1371/journal.pone.0181063.t001

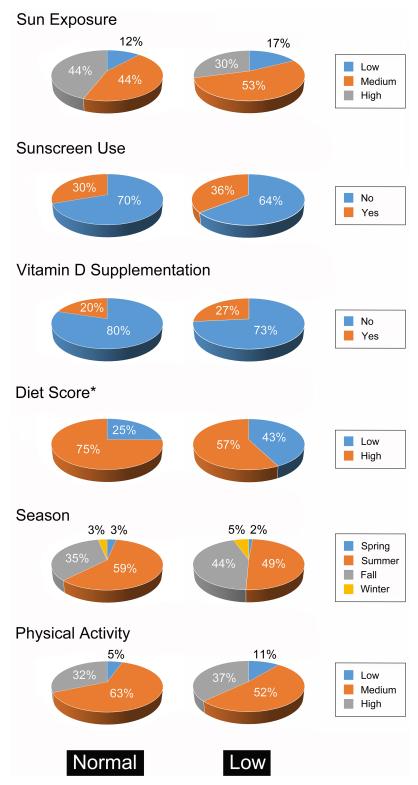


Fig 1. Comparison of factors affecting vitamin D level. Factors affecting vitamin D level between subjects (n = 170) with normal (n = 75) and low (n = 95) serum vitamin D level. *p < 0.005 between the two groups (Chi-square test).

https://doi.org/10.1371/journal.pone.0181063.g001



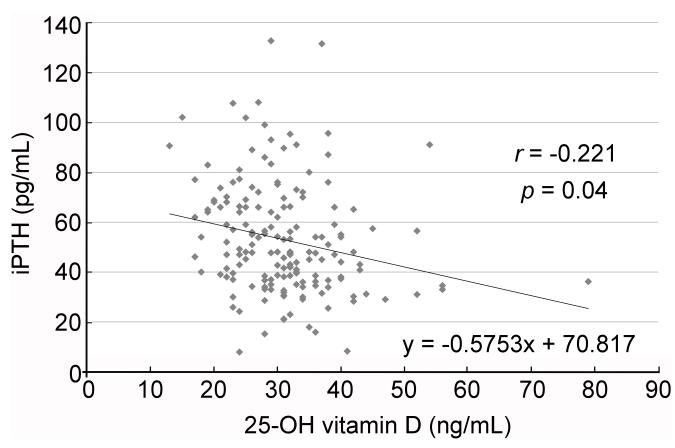


Fig 2. Relationship between serum parathyroid hormone (iPTH) concentration and 25-OH vitamin D level. Regression analysis with scatter plot showing inverse relationship between serum iPTH concentration and that of 25-OH vitamin D.

https://doi.org/10.1371/journal.pone.0181063.g002

ranging from 7.9 to 132.7 pg/mL. Hyperparathyroidism was found in 21.76% of all participants (37/170) in whom the prevalence of hyperparathyroidism was higher in the low vitamin D group (29.33%, 22/75) than that in the normal vitamin D group (15.8%, 15/95). Scattered plot (Fig 2) showed a negative correlation between 25-OH vitamin D level and iPTH (r = -0.221, p = 0.004) after adjustment for age and sex. No significant correlation was noted between serum iPTH and serum calcium levels (r = 0.013, p = 0.865).

Factor associated with low vitamin D status and gender differences

Chi-square test demonstrated significantly higher sun exposure in men than that in women (p = 0.003). The results of logistic regression of presumed risk factors for low vitamin D status were summarized in Table 2. Female gender was at higher risk of low vitamin D status (OR = 2.85, 95% CI: 1.39–5.85, p = 0.004) compared with that in their male counterparts. Low consumption of vitamin D-rich food was also significantly associated with an increased risk (OR = 2.09, 95% CI: 1.01–4.34, p = 0.049). In men (Table 3), inadequate intake of vitamin D-rich food was the only risk factor for a low vitamin D status (OR = 4.55, 95% CI: 1.43–14.43, p = 0.01, Nagelkerke $r^2 = 0.217$). However, in women (Table 4), a lack of sun exposure was the only predictable risk with dose-response relationship. Compared with their female counterparts with high extent of sun exposure, elderly women with low sun exposure (OR = 6.84, 95%)

	Odds ratio	95% CI		P value
Age	0.98	0.91	1.04	0.467
Gender				
Female	2.85	1.39	5.85	0.004
Male	1.00			
BMI	0.96	0.86	1.07	0.445
Sun exposure				
Low	1.39	0.46	4.19	0.561
Medium	1.71	0.79	3.70	0.176
High	1.00			
Sun screen				
No	1.28	0.58	2.84	0.541
Yes	1.00			
Vitamin D supplement				
No	0.75	0.32	1.75	0.504
Yes	1.00			
Diet score				
Low	2.09	1.01	4.34	0.049
High	1.00			
Season				
Spring	0.20	0.01	3.66	0.277
Summer	0.59	0.11	3.23	0.541
Fall	0.68	0.12	3.78	0.662
Winter	1.00			
Physical activity				
Low	1.14	0.27	4.77	0.859
Medium	0.53	0.25	1.11	0.092
High	1.00			

Table 2. Logistic regression for risk factors of low vitamin D status.

https://doi.org/10.1371/journal.pone.0181063.t002

CI: 1.40–33.51, p = 0.018) and moderate sun exposure (OR = 6.67, 95% CI: 1.79–24.77, p = 0.005) were at greater risks of low vitamin D status (Nagelkerke $r^2 = 0.252$).

Discussion

Although vitamin D deficiency and insufficiency are common worldwide, only a few studies have focused on the vitamin D status of the elderly. Moreover, limited surveys were completed in Asian countries so far. One multicenter survey showed a high prevalence of vitamin D deficiency in large cities in China, but no associated risk factors were reported [30]. Accordingly, our survey was designed to explore the risk factors of low vitamin D status in the elderly living in rural areas of Taiwan. Our survey demonstrated an incidence of low vitamin D status up to 44.1% in this population with female predominance (women: 57.7%, men: 30.6%).

Geographically, the percentage of vitamin D deficiency and insufficiency was higher than expectation in Asian countries, especially in South Asia [18]. In a multicenter vitamin D survey in China, the prevalence of vitamin D deficiency (<20 ng/mL) and vitamin D insufficiency (20–30 ng/mL) was 55.9% and 38.7%, respectively [30]. Another national survey in temperate areas of China reported the prevalence of vitamin D deficiency and insufficiency was 69.2 and 24.4%, respectively [25]. Based on the Korea National Health and Nutrition Examination Survey (KNHANES) 2008, Fourth Korea National Health and Nutrition Examination Surveys

	Odds ratio 0.99	95%CI		P value
Age		0.89	1.10	0.825
BMI	0.91	0.77	1.08	0.292
Sun exposure				
Low	0.24	0.02	2.98	0.264
Medium	0.57	0.18	1.80	0.335
High	1.00			
Sun screen				
No	0.91	0.26	3.16	0.884
Yes	1.00			
Vitamin D supplement				
No	0.73	0.18	3.03	0.663
Yes	1.00			
Diet score				
Low	4.55	1.43	14.43	0.010
High	1.00			
Season				
Spring	0.16	0.01	5.46	0.310
Summer	0.14	0.01	1.67	0.119
Fall	0.26	0.02	3.41	0.307
Winter	1.00			
Physical activity				
Low	0.47	0.44	0.05	4.125
Medium	0.53	0.70	0.22	2.160
High	1.00			

Table 3. Logistic regression for risk factors of low vitamin D status (Male).

https://doi.org/10.1371/journal.pone.0181063.t003

showed the prevalence of vitamin D deficiency was 47% in the males and 65% in the females [24]. As sun exposure is an essential source of vitamin D production, people living in low-latitude countries might be expected to have higher vitamin D levels than those living at high latitudes [1]. In contrast with China and Korea, Thailand, Taiwan and India are located at a lower latitude. However, the prevalence of low vitamin D status in people living in urban areas was reported to be up to 46.7% in one Thai study. For instance, the situation was more common in Bangkok (64.6%) than that in rural areas (33.5%), highlighting the impact of lifestyle and environmental factors on ultraviolet exposure and vitamin D synthesis [28]. In Taiwan, a survey including 215 participants living in Northern island demonstrated an incidence of 25-(OH)D deficiency up to 31% [34]. Even in tropical regions with plenty of sunshine such as India, more than 70% of the population suffered from vitamin D deficiency in all age groups and both genders [35].

Generally speaking, individual differences are significant contributors to vitamin D status. Vitamin D deficiency has been reported to be highly prevalent in pregnant women, children, older people, and residents of institutions [36]. Besides, more than 50% of postmenopausal women taking medication for osteoporosis also had 25-(OH)D \leq 30ng/mL [37, 38]. Despite being a notorious culprit responsible for skin pigmentation, ultraviolet B triggers vitamin D production [16, 39]. Several studies have already revealed a lower vitamin D status in Asian women than that in their male counterparts [25, 40–42]. The finding may be explained by the observation that Asian women generally prefer indoor activities and take more precautions against sunlight exposure owing to aesthetic concern. The more frequent use of sunscreens in

	Odds ratio 0.95	95%CI		P value
Age		0.85	1.07	0.371
BMI	0.93	0.78	1.11	0.421
Sun exposure				
Low	6.84	1.40	33.51	0.018
Medium	6.67	1.79	24.77	0.005
High	1.00			
Sun screen				
No	1.78	0.52	6.03	0.357
Yes	1.00			
Vitamin D supplement				
No	1.00	0.29	3.44	1.000
Yes	1.00			
Diet score				
Low	1.16	0.40	3.33	0.788
High	1.00			
Season				
Spring	0.000	0.00		1.000
Summer	1.22	0.07	20.78	0.891
Fall	0.68	0.05	9.09	0.769
Winter	1.00			
Physical activity				
Low	2.88	0.23	36.01	0.412
Medium	0.65	0.19	2.24	0.498
High	1.00			

Table 4. Logistic regression for risk factors of low vitamin D status (Female).

https://doi.org/10.1371/journal.pone.0181063.t004

females than in males may also contribute to the difference [43]. Our research also identified the female gender as a risk factor for a low vitamin D status. Although use of sunscreen was not a predictive factor in our survey, the extent of sun exposure was shown to be positively associated with a high vitamin D status in females. In comparison to the high sun exposure group, the medium sun exposure group (OR = 6.67) and low sun exposure group (OR = 6.84) had significantly increased risks for low vitamin D status. On the other hand, the association was not shown among men. One reason may be that more men obtained sufficient sun exposure in contrast to women (92.94% v.s.78.83%), which implied the influence of sun exposure on vitamin D status of men was not as crucial as that of women.

Although exposure to sunlight for 5–30 minutes daily was considered adequate for the production of pre-vitamin D3 in adults, old age is a significant predictor of low vitamin D status regardless of seasons or the extent of sun exposure [1, 44]. Indeed, based on skin biopsy samples, the capacity of production of pre-vitamin D3 in the elderly was less than half of that in young adults [26]. Besides, it has been reported that a 70-year-old person only produced onefourth of the amount of vitamin D compared with that of a 20-year-old through cutaneous synthesis [27]. This may partly account for the high prevalence of low vitamin D status in the rural areas of Taiwan, which is located in the subtropical region. Although the present study showed that the majority of the elderly population (85.89%) had adequate sun exposure because of farming, gardening, and other outdoor activities, they were not exempted from vitamin D sufficiency. The finding, therefore, may suggest a more detrimental influence of age on vitamin D status compared to that of inadequate sun exposure. Consumption of vitamin D-rich food, such as fortified milk, meat, oily fish, eggs, has been demonstrated to be limited in the diets of adolescents, regardless of races and genders [45–47]. Similarly, it has been reported that the elderly seldom take adequate daily amount to meet recommended daily allowance [1]. Moreover, for the aged population, in addition to the reduced absorption of dietary vitamin D associated with aging, polypharmacy commonly seen has also been shown to reduce absorption of vitamin D because of drug-food interaction [48]. Therefore, the importance of vitamin D supplementation increases by age. In our logistic regression model, only elderly men benefited from vitamin D-rich food to maintain adequate vitamin D status. The odds ratio of having a low vitamin D status in men with low diet score was 4.55 times higher than that in those with a high score. However, our results failed to show significant association between diet habits and serum vitamin D level among women. One possible explanation is that, unlike the case in men, factors other than diet such as menopausal status, lifestyle, and environmental influence may play a more important role in determining vitamin D status in women. [23, 49]

Predictors of vitamin D status were introduced in several large cohort studies, such as race, gender, body mass index, residential area, vitamin D supplement, dietary intake, and season [50, 51]. These possible indicators were all included in our logistic regression. In addition, gender was divided in subgroups to explore the difference between male and female living in the rural areas. Some previous studies, but not all, showed gender differences consistent with our results [52] [53]. Our data further revealed gender differences in predictors of low vitamin D status in the elderly. Moreover, the present study demonstrated that inadequate sun exposure and insufficient dietary vitamin D intake were significantly associated with low vitamin D status in women and men, respectively. The association between a low vitamin D status and malignancy has also been reported. Previous reviews [54, 55] showed a significant inverse relationship between colorectal cancer and vitamin D level, while the association in other malignancies including breast cancer, prostate cancer are less well defined [56, 57]. Another Asian study also supported the inverse relationship between vitamin D level and risk of rectal cancer [58]. In addition, previous epidemiological studies also showed that vitamin D level was negatively associated with cardiovascular risks, although benefit of vitamin D supplementation was not demonstrated in interventional studies [59]. On the other hand, another study reported an association between a low vitamin D status and the prevalence of osteoporosis, which was shown to improve with oral vitamin D supplementation [60]. The importance of gender influence on vitamin D status in the elderly as well as the role of vitamin D in the prevention and therapy of cancer, osteoporosis, cardiovascular diseases warrant further exploration [56, 61, 62].

Regarding the role of vitamin D in specific Taiwanese patient populations, there have been two studies investigating the impact of vitamin D level on disease progression. One of the studies investigated the influence of vitamin D status on bone complications in post-menopausal women [63], while the other studied the clinical significance of vitamin D level in patients with chronic hepatitis B [64]. The study focusing on vitamin D status and osteoporosis in 199 mid-dle-aged to elderly postmenopausal women demonstrated a prevalence of vitamin D inade-quacy (i.e., insufficiency and deficiency) of 86.6% [63]. Moreover, the other study on relatively young patients aged around 37 with chronic hepatitis B revealed a prevalence of vitamin D insufficiency and deficiency of 35% and 58%, respectively, both of which were shown to adversely affect prognostic outcome [64]. The two studies, therefore, highlight the high prevalence of vitamin D inadequacy in the diseased populations regardless of age and gender.

There are some limitations in the current study. Firstly, relatively young individuals were not recruited in the present study for evaluation of the impact of age on vitamin D status. Secondly, only subjects in rural areas were studied so that the vitamin D status of their counterparts in urban areas was unavailable for comparison. Thirdly, the information on some predictors that cannot be accurately quantitated, including dietary intake of vitamin D and sun exposure, was obtained through questionnaires that may have caused reporting and recall bias. Finally, the cross-sectional design of this study precluded the investigation of causa-tive effects of demographic and lifestyle factors on vitamin D status. Further cohort studies, therefore, are warranted to address these issues.

Conclusions

In conclusion, low vitamin D status, including vitamin D insufficiency and deficiency, was common in the aged population living in subtropical rural areas. Low sun exposure and inadequate dietary vitamin D consumption were associated with a status of low vitamin D in females and males, respectively.

Supporting information

S1 Table. Food frequency questionnaire (FFQ). English version of FFQ estimating oral vitamin D intake through assessing the frequency of vitamin D-rich food consumption in recent 3 months. (DOCX)

S2 Table. Food frequency questionnaire (FFQ). Original Chinese version of FFQ estimating oral vitamin D intake through assessing the frequency of vitamin D-rich food consumption in recent 3 months.

(DOCX)

S3 Table. Vitamin D contents of vitamin D-rich food items included in the food frequency questionnaire (FFQ). Vitamin D contents of commonly available vitamin D-rich food items included in FFQ. IU: International unit. (DOCX)

Author Contributions

Conceptualization: CHH CKS. Data curation: CHH YTAH CKS. Formal analysis: CHH YTAH CKS. Investigation: CHH YTAH CKS. Methodology: CHH CKS. Project administration: CHH YTAH YCL CKS. Resources: CHH CKS. Software: CHH YTAH YCL CKS. Supervision: CKS. Validation: CHH CKS. Visualization: CHH YCL CKS. Writing – original draft: CHH CKS. Writing – review & editing: CKS.

References

- Holick MF. Vitamin D deficiency. The New England journal of medicine. 2007; 357(3):266–81. Epub 2007/07/20. https://doi.org/10.1056/NEJMra070553 PMID: 17634462.
- Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. The American journal of clinical nutrition. 2006; 84(1):18–28. PMID: 16825677.
- DeLuca HF. Overview of general physiologic features and functions of vitamin D. The American journal of clinical nutrition. 2004; 80(6 Suppl):1689S–96S. PMID: 15585789.
- Penna G, Roncari A, Amuchastegui S, Daniel KC, Berti E, Colonna M, et al. Expression of the inhibitory receptor ILT3 on dendritic cells is dispensable for induction of CD4+Foxp3+ regulatory T cells by 1,25dihydroxyvitamin D3. Blood. 2005; 106(10):3490–7. https://doi.org/10.1182/blood-2005-05-2044 PMID: 16030186.
- Pfeifer M, Begerow B, Minne HW, Nachtigall D, Hansen C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. The Journal of clinical endocrinology and metabolism. 2001; 86(4):1633–7. https://doi.org/10.1210/jcem.86.4.7393 PMID: 11297596.
- Myrup B, Jensen GF, McNair P. Cardiovascular risk factors during estrogen-norethindrone and cholecalciferol treatment. Archives of internal medicine. 1992; 152(11):2265–8. PMID: 1332634.
- Scragg R, Khaw KT, Murphy S. Effect of winter oral vitamin D3 supplementation on cardiovascular risk factors in elderly adults. European journal of clinical nutrition. 1995; 49(9):640–6. PMID: 7498100.
- Bhan A, Rao AD, Rao DS. Osteomalacia as a result of vitamin D deficiency. Endocrinology and metabolism clinics of North America. 2010; 39(2):321–31, table of contents. https://doi.org/10.1016/j.ecl.2010. 02.001 PMID: 20511054.
- Christakos S, Dhawan P, Verstuyf A, Verlinden L, Carmeliet G. Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. Physiol Rev. 2016; 96(1):365–408. https://doi.org/10. 1152/physrev.00014.2015 PMID: 26681795.
- Holick MF. Resurrection of vitamin D deficiency and rickets. The Journal of clinical investigation. 2006; 116(8):2062–72. https://doi.org/10.1172/JCI29449 PMID: 16886050.
- Wranicz J, Szostak-Wegierek D. Health outcomes of vitamin D. Part II. Role in prevention of diseases. Rocz Panstw Zakl Hig. 2014; 65(4):273–9. PMID: 25526571.
- Bjelakovic G, Gluud LL, Nikolova D, Whitfield K, Wetterslev J, Simonetti RG, et al. Vitamin D supplementation for prevention of mortality in adults. Cochrane Database Syst Rev. 2011;(7):CD007470. https://doi.org/10.1002/14651858.CD007470.pub2 PMID: 21735411.
- Martineau AR, Cates CJ, Urashima M, Jensen M, Griffiths AP, Nurmatov U, et al. Vitamin D for the management of asthma. Cochrane Database Syst Rev. 2016; 9:CD011511. <u>https://doi.org/10.1002/14651858.CD011511.pub2</u> PMID: 27595415.
- Martineau AR, Jolliffe DA, Hooper RL, Greenberg L, Aloia JF, Bergman P, et al. Vitamin D supplementation to prevent acute respiratory tract infections: systematic review and meta-analysis of individual participant data. BMJ (Clinical research ed). 2017; 356:i6583. https://doi.org/10.1136/bmj.i6583 PMID: 28202713.
- Judd SE, Tangpricha V. Vitamin D deficiency and risk for cardiovascular disease. The American journal of the medical sciences. 2009; 338(1):40–4. Epub 2009/07/14. https://doi.org/10.1097/MAJ. 0b013e3181aaee91 PMID: 19593102.
- Holick MF. High prevalence of vitamin D inadequacy and implications for health. Mayo Clinic proceedings. 2006; 81(3):353–73. https://doi.org/10.4065/81.3.353 PMID: 16529140.
- Reid IR, Gallagher DJ, Bosworth J. Prophylaxis against vitamin D deficiency in the elderly by regular sunlight exposure. Age and ageing. 1986; 15(1):35–40. PMID: 3953329.
- Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2009; 20(11):1807–20. https://doi.org/10.1007/s00198-009-0954-6 PMID: 19543765.
- Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. Lancet. 1998; 351 (9105):805–6. PMID: 9519960.
- Adams JS, Hewison M. Update in vitamin D. The Journal of clinical endocrinology and metabolism. 2010; 95(2):471–8. https://doi.org/10.1210/jc.2009-1773 PMID: 20133466.
- Giovannucci E. Expanding roles of vitamin D. The Journal of clinical endocrinology and metabolism. 2009; 94(2):418–20. https://doi.org/10.1210/jc.2008-2695 PMID: 19193916.

- 22. Looker AC, Johnson CL, Lacher DA, Pfeiffer CM, Schleicher RL, Sempos CT. Vitamin D status: United States, 2001–2006. NCHS data brief. 2011;(59):1–8. PMID: 21592422.
- Mitchell DM, Henao MP, Finkelstein JS, Burnett-Bowie SA. Prevalence and predictors of vitamin D deficiency in healthy adults. Endocrine practice: official journal of the American College of Endocrinology and the American Association of Clinical Endocrinologists. 2012; 18(6):914–23. <u>https://doi.org/10.4158/EP12072.OR PMID: 22982792</u>.
- Choi HS, Oh HJ, Choi H, Choi WH, Kim JG, Kim KM, et al. Vitamin D insufficiency in Korea—a greater threat to younger generation: the Korea National Health and Nutrition Examination Survey (KNHANES) 2008. The Journal of clinical endocrinology and metabolism. 2011; 96(3):643–51. <u>https://doi.org/10.1210/jc.2010-2133</u> PMID: 21190984.
- Lu L, Yu Z, Pan A, Hu FB, Franco OH, Li H, et al. Plasma 25-hydroxyvitamin D concentration and metabolic syndrome among middle-aged and elderly Chinese individuals. Diabetes care. 2009; 32(7):1278– 83. https://doi.org/10.2337/dc09-0209 PMID: 19366976.
- MacLaughlin J, Holick MF. Aging decreases the capacity of human skin to produce vitamin D3. The Journal of clinical investigation. 1985; 76(4):1536–8. <u>https://doi.org/10.1172/JCI112134</u> PMID: 2997282.
- Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. Lancet. 1989; 2(8671):1104– 5. PMID: 2572832.
- Chailurkit LO, Aekplakorn W, Ongphiphadhanakul B. Regional variation and determinants of vitamin D status in sunshine-abundant Thailand. BMC public health. 2011; 11:853. https://doi.org/10.1186/1471-2458-11-853 PMID: 22074319.
- Nimitphong H, Holick MF. Vitamin D status and sun exposure in southeast Asia. Dermato-endocrinology. 2013; 5(1):34–7. https://doi.org/10.4161/derm.24054 PMID: 24494040.
- Yu S, Fang H, Han J, Cheng X, Xia L, Li S, et al. The high prevalence of hypovitaminosis D in China: a multicenter vitamin D status survey. Medicine. 2015; 94(8):e585. https://doi.org/10.1097/MD. 000000000000585 PMID: 25715263.
- Gordon CM, DePeter KC, Feldman HA, Grace E, Emans SJ. Prevalence of vitamin D deficiency among healthy adolescents. Archives of pediatrics & adolescent medicine. 2004; 158(6):531–7. Epub 2004/06/ 09. https://doi.org/10.1001/archpedi.158.6.531 PMID: 15184215.
- 32. Płudowski P, Karczmarewicz E, Bayer M, Carter G, Chlebna-Sokół D, Czech-Kowalska J, et al. Practical guidelines for the supplementation of vitamin D and the treatment of deficits in Central Europe—recommended vitamin D intakes in the general population and groups at risk of vitamin D deficiency. Endokrynologia Polska. 2013; 64(4):319–27. https://doi.org/10.5603/ep.2013.0012 PMID: 24002961
- Liou YM, Jwo CJ, Yao KG, Chiang LC, Huang LH. Selection of appropriate Chinese terms to represent intensity and types of physical activity terms for use in the Taiwan version of IPAQ. J Nurs Res. 2008; 16(4):252–63. PMID: 19061172.
- Chang CI, Chan DC, Kuo KN, Hsiung CA, Chen CY. Vitamin D insufficiency and frailty syndrome in older adults living in a Northern Taiwan community. Arch Gerontol Geriatr. 2010; 50 Suppl 1:S17–21. https://doi.org/10.1016/S0167-4943(10)70006-6 PMID: 20171450.
- Babu US, Calvo MS. Modern India and the vitamin D dilemma: evidence for the need of a national food fortification program. Molecular nutrition & food research. 2010; 54(8):1134–47. <u>https://doi.org/10.1002/</u> mnfr.200900480 PMID: 20440690.
- van Schoor NM, Lips P. Worldwide vitamin D status. Best Pract Res Clin Endocrinol Metab. 2011; 25(4):671–80. https://doi.org/10.1016/j.beem.2011.06.007 PMID: 21872807.
- Holick MF, Siris ES, Binkley N, Beard MK, Khan A, Katzer JT, et al. Prevalence of Vitamin D inadequacy among postmenopausal North American women receiving osteoporosis therapy. The Journal of clinical endocrinology and metabolism. 2005; 90(6):3215–24. https://doi.org/10.1210/jc.2004-2364 PMID: 15797954.
- Lips P, Hosking D, Lippuner K, Norquist JM, Wehren L, Maalouf G, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. Journal of internal medicine. 2006; 260(3):245–54. <u>https://doi.org/10.1111/j.1365-2796.2006.01685.x</u> PMID: 16918822.
- Holick MF. Vitamin D: its role in cancer prevention and treatment. Progress in biophysics and molecular biology. 2006; 92(1):49–59. Epub 2006/03/29. <u>https://doi.org/10.1016/j.pbiomolbio.2006.02.014</u> PMID: 16566961.
- Robien K, Butler LM, Wang R, Beckman KB, Walek D, Koh WP, et al. Genetic and environmental predictors of serum 25-hydroxyvitamin D concentrations among middle-aged and elderly Chinese in Singapore. Br J Nutr. 2013; 109(3):493–502. https://doi.org/10.1017/S0007114512001675 PMID: 22583563.

- Moy FM. Vitamin D status and its associated factors of free living Malay adults in a tropical country, Malaysia. J Photochem Photobiol B. 2011; 104(3):444–8. https://doi.org/10.1016/j.jphotobiol.2011.05. 002 PMID: 21636288.
- Kung AW, Lee KK. Knowledge of vitamin D and perceptions and attitudes toward sunlight among Chinese middle-aged and elderly women: a population survey in Hong Kong. BMC public health. 2006; 6:226. https://doi.org/10.1186/1471-2458-6-226 PMID: 16956420.
- Holick MF. McCollum Award Lecture, 1994: vitamin D—new horizons for the 21st century. The American journal of clinical nutrition. 1994; 60(4):619–30. PMID: 8092101.
- 44. Rucker D, Allan JA, Fick GH, Hanley DA. Vitamin D insufficiency in a population of healthy western Canadians. CMAJ: Canadian Medical Association journal = journal de l'Association medicale canadienne. 2002; 166(12):1517–24. Epub 2002/06/21. PMID: 12074117.
- Yu A, Kim J, Kwon O, Oh SY, Kim J, Yang YJ. The association between serum 25-hydroxyvitamin d concentration and consumption frequencies of vitamin d food sources in korean adolescents. Clin Nutr Res. 2013; 2(2):107–14. https://doi.org/10.7762/cnr.2013.2.2.107 PMID: 23908977.
- 46. Van Horn LV, Bausermann R, Affenito S, Thompson D, Striegel-Moore R, Franko D, et al. Ethnic differences in food sources of vitamin D in adolescent American girls: the National Heart, Lung, and Blood Institute Growth and Health Study. Nutr Res. 2011; 31(8):579–85. <u>https://doi.org/10.1016/j.nutres.</u> 2011.07.003 PMID: 21925342.
- Holick MF, Shao Q, Liu WW, Chen TC. The vitamin D content of fortified milk and infant formula. The New England journal of medicine. 1992; 326(18):1178–81. https://doi.org/10.1056/ NEJM199204303261802 PMID: 1313548.
- Lo CW, Paris PW, Clemens TL, Nolan J, Holick MF. Vitamin D absorption in healthy subjects and in patients with intestinal malabsorption syndromes. The American journal of clinical nutrition. 1985; 42 (4):644–9. PMID: 4050723.
- Aggarwal S, Nityanand. Calcium and vitamin D in post menopausal women. Indian J Endocrinol Metab. 2013; 17(Suppl 3):S618–20. https://doi.org/10.4103/2230-8210.123549 PMID: 24910823.
- Giovannucci E, Liu Y, Rimm EB, Hollis BW, Fuchs CS, Stampfer MJ, et al. Prospective study of predictors of vitamin D status and cancer incidence and mortality in men. J Natl Cancer Inst. 2006; 98(7):451– 9. https://doi.org/10.1093/jnci/djj101 PMID: 16595781.
- Liu E, Meigs JB, Pittas AG, Economos CD, McKeown NM, Booth SL, et al. Predicted 25-hydroxyvitamin D score and incident type 2 diabetes in the Framingham Offspring Study. The American journal of clinical nutrition. 2010; 91(6):1627–33. https://doi.org/10.3945/ajcn.2009.28441 PMID: 20392893.
- Vierucci F, Del Pistoia M, Fanos M, Erba P, Saggese G. Prevalence of hypovitaminosis D and predictors of vitamin D status in Italian healthy adolescents. Italian journal of pediatrics. 2014; 40:54. <u>https:// doi.org/10.1186/1824-7288-40-54 PMID: 24902694.</u>
- Al-Musharaf S, Al-Othman A, Al-Daghri NM, Krishnaswamy S, Yusuf DS, Alkharfy KM, et al. Vitamin D deficiency and calcium intake in reference to increased body mass index in children and adolescents. Eur J Pediatr. 2012; 171(7):1081–6. https://doi.org/10.1007/s00431-012-1686-8 PMID: 22311168.
- Lee JE, Li H, Chan AT, Hollis BW, Lee IM, Stampfer MJ, et al. Circulating levels of vitamin D and colon and rectal cancer: the Physicians' Health Study and a meta-analysis of prospective studies. Cancer Prev Res (Phila). 2011; 4(5):735–43. <u>https://doi.org/10.1158/1940-6207.CAPR-10-0289</u> PMID: 21430073.
- 55. Ma Y, Zhang P, Wang F, Yang J, Liu Z, Qin H. Association between vitamin D and risk of colorectal cancer: a systematic review of prospective studies. J Clin Oncol. 2011; 29(28):3775–82. https://doi.org/10. 1200/JCO.2011.35.7566 PMID: 21876081.
- Garland CF, Garland FC, Gorham ED, Lipkin M, Newmark H, Mohr SB, et al. The role of vitamin D in cancer prevention. American journal of public health. 2006; 96(2):252–61. Epub 2005/12/29. https://doi. org/10.2105/AJPH.2004.045260 PMID: 16380576.
- 57. Mondul AM, Weinstein SJ, Layne TM, Albanes D. Vitamin D and Cancer Risk and Mortality: State of the Science, Gaps, and Challenges. Epidemiol Rev. 2017:1–21.
- Otani T, Iwasaki M, Sasazuki S, Inoue M, Tsugane S, Japan Public Health Center-Based Prospective Study G. Plasma vitamin D and risk of colorectal cancer: the Japan Public Health Center-Based Prospective Study. Br J Cancer. 2007; 97(3):446–51. https://doi.org/10.1038/sj.bjc.6603892 PMID: 17622244.
- Grubler MR, Marz W, Pilz S, Grammer TB, Trummer C, Mullner C, et al. Vitamin-D concentrations, cardiovascular risk and events—a review of epidemiological evidence. Rev Endocr Metab Disord. 2017. https://doi.org/10.1007/s11154-017-9417-0 PMID: 28451877.
- 60. Weaver CM, Alexander DD, Boushey CJ, Dawson-Hughes B, Lappe JM, LeBoff MS, et al. Calcium plus vitamin D supplementation and risk of fractures: an updated meta-analysis from the National

Osteoporosis Foundation. Osteoporosis international: a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2016; 27(1):367–76. https://doi.org/10.1007/s00198-015-3386-5 PMID: 26510847.

- Chapuy MC, Arlot ME, Duboeuf F, Brun J, Crouzet B, Arnaud S, et al. Vitamin D3 and calcium to prevent hip fractures in the elderly women. The New England journal of medicine. 1992; 327(23):1637–42. Epub 1992/12/03. https://doi.org/10.1056/NEJM199212033272305 PMID: 1331788.
- **62.** Bulathsinghala P, Syrigos KN, Saif MW. Role of vitamin d in the prevention of pancreatic cancer. Journal of nutrition and metabolism. 2010; 2010:721365. Epub 2011/01/29. <u>https://doi.org/10.1155/2010/721365</u> PMID: 21274445.
- Hwang JS, Tsai KS, Cheng YM, Chen WJ, Tu ST, Lu KH, et al. Vitamin D status in non-supplemented postmenopausal Taiwanese women with osteoporosis and fragility fracture. BMC Musculoskelet Disord. 2014; 15:257. https://doi.org/10.1186/1471-2474-15-257 PMID: 25069806.
- Chan HL, Elkhashab M, Trinh H, Tak WY, Ma X, Chuang WL, et al. Association of baseline vitamin D levels with clinical parameters and treatment outcomes in chronic hepatitis B. J Hepatol. 2015; 63 (5):1086–92. https://doi.org/10.1016/j.jhep.2015.06.025 PMID: 26143444.