Clinical and demographic predictors for vitamin D deficiency in multiethnic Asian patients with chronic kidney disease

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Abstract
Background. Vitamin D deficiency is common in patients with chronic kidney disease (CKD) and can cause skeletal and extraskeletal complications. The purpose of this study is to determine the clinical and demographic risk factors for vitamin D deficiency in multiethnic CKD patients in Singapore, a sun-rich country, so that patients at risk can be identified and treated early.

Methods. Pre-dialysis CKD patients from the National University Hospital (NUH), Singapore, Outpatient Renal Clinic who had their serum 25-hydroxyvitamin D [25(OH)D] levels measured between January 2008 and October 2010 were included. Their clinical and demographic parameters were collected from hospital databases and medical charts. Logistic regression was used to identify potential predictors for vitamin D deficiency in these patients. Two models, Mt30 and Mt16, were built using threshold serum 25(OH)D levels of ≤30 and <16 ng/mL, respectively.

Results. Of the 219 patients included, 82.7 and 25.6% had serum 25(OH)D levels ≤30 and <16 ng/mL, respectively. Predictors identified for vitamin D deficiency include absence of vitamin D supplementation, type 2 diabetes mellitus (DM), non-cancer diagnosis, younger age, Malay race, treatment with calcitriol and higher serum bicarbonate (CO2) levels. Common predictors for the two models were lack of vitamin D supplementation and DM. The areas under the receiver-operating characteristic (ROC) curve for the validation sets were 0.697 and 0.687 for the Mt30 and Mt16 models, respectively.

Conclusions. Vitamin D deficiency is common among multiethnic CKD patients in Singapore. Risk factors identified in this study include absence of vitamin D supplementation, DM, non-cancer diagnosis, young age, Malay race, calcitriol treatment and higher serum CO2. The knowledge of these risk factors is useful for predicting vitamin D deficiency in CKD patients in Singapore.

Keywords: chronic kidney disease; mineral and bone disorder; risk factors; vitamin D deficiency; 25-hydroxyvitamin D

Introduction
Vitamin D deficiency is common in patients with chronic kidney disease (CKD), and the incidence is higher in later stages of CKD [1]. Vitamin D plays an important role in bone and mineral homeostasis, which is known as the classical actions of vitamin D [2]. The classic target organs of vitamin D include intestine, bone, kidneys and parathyroid glands. Non-classical actions of vitamin D have also been elicited in the immune system, renin-angiotensin–aldosterone system, heart muscles and arterial walls [2]. Vitamin D deficiency can result in several complications, such as secondary hyperparathyroidism, low bone mineral density, cardiovascular and autoimmune diseases [3].

Vitamin D is available in two forms: vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D2 is available from plant sterols, while vitamin D3 is derived from sunlight, diet and fortified foods (synthetic) [4]. Under ultraviolet (UV) B radiation from sunlight, 7-dehydrocholesterol is converted to pre-vitamin D3 in the skin [4]. Both forms of vitamin D are subsequently converted to the active form by undergoing two hydroxylation steps [4]. The first hydroxylation occurs in the liver to form 25-hydroxyvitamin D2 and D3 [25(OH)D2 and D3], which are in turn converted into the biologically active compound, 1,25-dihydroxyvitamin D3 [1,25(OH)2D3], (calcitriol) by hydroxylation in the kidney [2, 4].

Serum 25-hydroxyvitamin D [25(OH)D] concentration is indicative of vitamin D stores in the body. Patients with CKD are more likely to have lower levels of 25(OH)D than those without kidney disease. In a study conducted in Boston, USA, kidney disease was found to be a major risk factor for low serum 25(OH)D in hospitalized patients [5]. Reasons for CKD patients developing vitamin D deficiency
could include reduction in sunlight exposure due to inactivity and lower intake of vitamin D-rich foods [6]. Furthermore, in CKD patients with proteinuria, urinary loss of vitamin D-binding proteins (DBP) is high [6].

A recent study conducted in dialysis patients in the USA identified black race, female sex, winter season, and hypoalbuminemia as predictors of vitamin D deficiency [7]. Another cross-sectional study involving 1026 pre-dialysis CKD patients of any stage revealed that low glomerular filtration rate (GFR) (<30 mL/min/1.73 m²), winter season, obesity, proteinuria, hypoalbuminemia, diabetes mellitus (DM) and hypertension were associated with vitamin D deficiency [8]. To date, similar analyses have not been conducted in the Asian CKD patient population. The Asian population is different from the US population due to differences in geographical location and the climate in which they live. In addition, the amount of sunlight exposure, skin color and genetics are also different. Thus, the potential risk factors of vitamin D deficiency in Asian CKD patients could be different.

Currently, the risk factors for vitamin D deficiency in CKD patients in Singapore (1°22’ north of the equator), a sun-rich country, are unknown. As such, the aim of this study is to identify clinical and demographic parameters which are potential risk factors for vitamin D deficiency in multiethnic pre-dialysis patients in Singapore. These identified predictors will be useful to clinicians for the prediction of vitamin D deficiency, so that replacement with vitamin D supplements can be initiated early in CKD patients, before their kidney function deteriorates. Specifically, the primary objective of this study was to determine if there are any routinely measured clinical and demographic parameters that can predict vitamin D deficiency in CKD patients. The secondary objective was to build a model that can predict the likelihood of vitamin D deficiency in CKD patients.

Materials and methods

This was a single-center, retrospective, cross-sectional, observational study approved by the National Healthcare Group Domain-Specific Review Boards which is the local institutional review board. Patients from the National University Hospital (NUH), Singapore, Outpatient Renal Clinic who had their serum creatinine and 25(OH)D levels measured between January 2008 and October 2010 were included in this study. These patients were stratified by stages of CKD from 1 to 5, by estimating the GFR using the modification of diet in renal disease equation for standardized creatinine. Stage 5 CKD patients receiving renal replacement therapy (dialysis or renal transplant) were excluded.

The patients were classified into different categories of vitamin D deficiency based on their serum 25(OH)D concentrations. The classification system was based on that published in the US National Kidney Foundation Kidney Dialysis Outcomes Quality Initiative (KDOQI) guidelines [6].

Patient clinical and demographic parameters were collected from their electronic medical records and case notes. These parameters included demographics, clinical and laboratory data, as well as medical and medication histories. The laboratory data and medication histories were collected from the date closest to the date of serum 25(OH)D measurement.

Pre-processing of data

In this study, the entire dataset was split into two sets: a training set for developing a model to determine the likelihood of vitamin D deficiency in pre-dialysis patients and a validation set to validate the model. The training set comprised of 169 patients whose serum 25(OH)D levels were measured from January 2008 to mid-June 2010, while the validation set consisted of 50 patients whose serum 25(OH)D levels were measured from mid-June 2010 to October 2010. This strategy of dividing the dataset according to time was to ensure that the validation set would be able to reliably assess the historical generalizability of the final model [9].

A threshold serum 25(OH)D level was used to split the patients into two groups: vitamin D-deficient and non-vitamin D-deficient groups for each model. The aim of using a threshold is to identify CKD patients who are more likely to be vitamin D deficient, so that replacement with vitamin D supplements can be initiated early. The threshold serum 25(OH)D levels used were ≤30 and <16 ng/mL for models M_{130} and M_{16}, respectively. These thresholds were selected based on the KDOQI guidelines [6] classification of vitamin D deficiency where 25(OH)D concentration of 30 ng/mL is the cutoff for vitamin D sufficiency and deficiency/insufficiency, and 16 ng/mL is the cutoff for vitamin D insufficiency and deficiency.

Identification of predictors for vitamin D deficiency and model development

Logistic regression analyses were used to estimate the crude odds ratio (OR) for the available patient clinical and demographic parameters. Parameters with statistically significant (P < 0.05) crude ORs were identified as potential predictors and were used to construct the multivariate logistic regression models M_{130} and M_{16}. The adjusted ORs for these predictors were then estimated from M_{130} and M_{16}. In these analyses, 25(OH)D levels >30 and ≥16 ng/mL were used as the reference categories, respectively. The classification cutoffs for both logistic regression models were adjusted based on the proportion of patients with vitamin D deficiency. This was done to prevent the models from having bias predictions towards the majority class. There were 137 patients with 25(OH)D levels ≤30 ng/mL in the training set. Thus, the classification cutoff for the M_{130} model was 137/169, which was ~0.81. For the M_{16} model, there were 40 patients with 25(OH)D levels <16 ng/mL in the training set. Thus, the classification cutoff for M_{16} was 40/169, which was ~0.24. Statistical analyses were performed using IBM SPSS Statistics, version 19.

Performance evaluation of model

The prediction performance of models M_{130} and M_{16} was assessed using the training set and the validation set. In this study, the validation set was used only once in the entire study; in the validation step. This simulates a prospective study in a way where the model was validated using the validation set which was comprised of patients who had their serum 25(OH)D levels measured at a later part of the study period (mid-June to October 2010).
Vitamin D sufficiency, vitamin D insufficiency, and vitamin D deficiency were identified. Of these, 16.6% had serum 25(OH)D levels ≤16 ng/mL for models M130 and M116, respectively. Thus, both M130 and M116 had comparable performance. The model performances of models M130 and M116 for the validation sets are shown in Table 5. The positive class for this study refers to patients with vitamin D deficiency and insufficiency, while the negative class refers to patients with vitamin D sufficiency.

Discussion

This study identified patients not taking any vitamin D supplements and those with type 2 DM as predictors for vitamin D insufficiency or deficiency.

Patients not receiving vitamin D supplementation, either ergocalciferol or cholecalciferol, had a higher risk of vitamin D deficiency. As shown in Tables 3 and 4, this predictor was a common predictor in both models. This predictor was significant and indicates that replacement with ergo/cholecalciferol may reduce the likelihood of vitamin D insufficiency or deficiency. Studies have also shown that vitamin D supplementation using ergocalciferol and cholecalciferol can increase serum levels of 25(OH)D in CKD patients [4, 10]. In our study, 82.7% of the
early-stage CKD or a screening program for vitamin D deficiency and subsequent treatment of deficient patients would result in better outcomes from pharmacoeconomic and clinical standpoints requires further investigation. Nonetheless, considering the high prevalence and ease of detection of vitamin D insufficiency or deficiency, as well as the effectiveness, safety and relative affordability of treatment, suboptimal vitamin D status should be treated and its sufficiency maintained thereafter. Type 2 DM was also associated with an increased prevalence of vitamin D deficiency in this study and was predicted by both models used in this study. DM was also identified as a predictor in the study by Bhan et al. [7] where two of its models had similar threshold serum 25(OH)D levels as this study. The association between vitamin D deficiency and type 2 DM has also been observed in several other studies [8,14–16]. However, the exact cause-and-effect relationship between vitamin D deficiency and type 2 DM has not been proven in clinical studies.

Race was found to be a predictor for vitamin D deficiency in one of the models. Singapore has a multi-ethnic population comprising of Chinese, Malays, Indians and other minority races. Both univariate and multivariate logistic regression analyses identified Malay race as a predictor for vitamin D deficiency. As skin pigmentation is commonly regarded as a reason for reduced vitamin D synthesis, this result was not unexpected. Malays and Indians have a darker skin color and thus have a greater amount of melanin. Melanin absorbs the solar UVB radiation and affects the synthesis of pre-vitamin D$_3$ from 7-dehydrocholesterol in the skin [17]. Therefore, the production of pre-vitamin D$_3$ is greatly reduced in darker-skinned individuals [17]. In fact, our results are in concordance with the study carried out by Bhan et al. [7] which had identified black race as one of the predictors for vitamin D deficiency in the US population. However, it was surprising that only the Malay race but not Indian was significantly associated with vitamin D deficiency, as individuals of both racial groups have darker skin compared with Chinese.

A recent study [18] comparing dark- versus fair-skinned individuals with comparable baseline vitamin D status found no significant differences in the change in 25(OH)D levels after UVB exposure. The authors thus concluded that skin pigmentation is not related to vitamin D deficiency. Indeed, the exact role of skin pigmentation in vitamin D synthesis after sun exposure is unclear as conflicting results have been shown. In this study, the higher ORs of vitamin D deficiency in Malays could be attributed to cultural, lifestyle and clothing differences. For example, most Malay women follow a custom in their dressing that covers most of their skin. In fact, this has been identified as an independent factor of vitamin D deficiency in the Middle East and some parts of South-East Asia [19].

In the present study, the OR of a patient having vitamin D insufficiency or deficiency [25(OH)D $\leq$30 ng/mL] increases by 1.04-fold with every decrease in 1 year of age. This is equivalent to a 1.54-fold increase in risk with every decrease in 10 years of age in the M$_{30}$ model. This was rather unusual as dermal synthesis of pre-vitamin D$_3$ has been shown to be reduced in older patients [20]. As such, older patients would have been expected to be at a higher risk of vitamin D deficiency compared with younger patients. On the other hand, younger patients from our study population could have been better educated about the preventive measures of
cardiovascular disease and anemia needs to be done to determine the association.

Serum bicarbonate levels, its association with vitamin D deficiency, type 2 DM, race, treatment with calcitriol and other covariates such as vitamin D supplementation were also identified in univariate analysis. After adjustment for other covariates such as vitamin D supplementation, type 2 DM, race, treatment with calcitriol and serum bicarbonate levels, its association with vitamin D deficiency failed to achieve statistical significance. Thus, more work needs to be done to determine the association between the female gender and vitamin D deficiency in our local CKD population.

Predictors for vitamin D deficiency identified in other studies include the measured GFR, cardiovascular disease and anemia. However, in our study, the crude ORs for these parameters (CKD stage, presence of cardiovascular disease and anemia) were not found to be statistically significant (Table 6). In the study involving 1026 predialysis CKD patients conducted by Urena et al., a decline in the measured glomerular filtration rate (GFR) was independently associated with vitamin D deficiency.

Though this was not observed in this study as serum creatinine, CKD stage and estimated GFR were all not found to be associated with vitamin D status. A possible reason for the lack of association is the small sample size of this study. Thus, these parameters were not considered for both M₃₀ and M₁₆₆ models.

In recent years, genome-wide association studies that examined the influence of genetic variations on the vitamin D status have been prevalent. These studies included populations from North America, Canada and Europe and showed that variants in GC (gene encoding vitamin D binding protein), DHR7 (responsible for removing pre-cholesterol from the vitamin D pathway thus reducing the availability of a substrate for 25(OH)D), CYP2R1 (responsible for hydroxylation of the vitamin D precursor to 25(OH)D), CYP24A1 (responsible for 24-hydroxylation, degradation and excretion of 25(OH)D) and CYP27B1 (responsible for conversion of 25(OH)D to the active 1,25(OH)₂D) affect 25(OH)D concentrations. However, these genetic effects and their corresponding associations with the vitamin D status in the Asian population have not been investigated and thus warrant further research.

### Model performance

Validation sets for M₃₀ and M₁₆₆ had AUCs of 0.697 and 0.687, respectively. These AUCs were comparable with that of the US study conducted by Bhan et al. using similar threshold serum 25(OH)D levels and logistic regression algorithm.

### Limitations

A potential limitation of our study is the relatively small sample size of 219 CKD patients. Due to the lack of complete clinical, laboratory and medications information for some patients, certain clinically relevant parameters had to be excluded from model development. For example, the clinical data of height and weight were missing for 25 patients. Under the laboratory data, not all patients had their serum levels of corrected calcium and i-PTH measured. Additionally, total cholesterol and urinary protein concentrations were not collected. Nonetheless, it would be possible to reconstruct the model with the inclusion of these parameters when more information becomes available in the future.

### Table 5. Model performance for validation sets

| Parameter | True Positive | False Positive | True Negative | False Negative | Sensitivity | Specificity | Accuracy |
|-----------|---------------|----------------|---------------|----------------|-------------|------------|----------|
| Model M₃₀ | 36            | 4              | 2             | 8              | 81.8        | 33.3       | 76.0     |
| Model M₁₆₆| 13            | 17             | 17            | 3              | 81.3        | 50.0       | 60.0     |

### Table 6. Odds ratio of vitamin D deficiency by covariates found to be important in other studies

| Parameter                  | True Positive | False Positive | True Negative | False Negative | Crude OR (95% CI) for 25(OH)D <30 ng/mL | Crude OR (95% CI) for 25(OH)D <16 ng/mL |
|----------------------------|---------------|----------------|---------------|----------------|----------------------------------------|----------------------------------------|
| CKD                        |               |                |               |                |                                        |                                        |
| Stage 1                    | 1.00 (reference) | 1.00 (reference) |               |                |                                        |                                        |
| Stage 2                    | 2.50 (0.15–4.28) | 0.67 (0.04–10.25) |               |                |                                        |                                        |
| Stage 3                    | 4.33 (0.34–55.21) | 0.37 (0.03–4.49) |               |                |                                        |                                        |
| Stage 4                    | 1.62 (0.14–18.96) | 0.72 (0.06–8.37) |               |                |                                        |                                        |
| Stage 5                    | 1.50 (0.12–19.64) | 1.00 (0.08–12.76) |               |                |                                        |                                        |
| Anemia                     |               |                |               |                |                                        |                                        |
| No                         | 1.00 (reference) | 1.00 (reference) |               |                |                                        |                                        |
| Yes                        | 1.31 (0.57–2.97) | 1.87 (0.91–3.86) |               |                |                                        |                                        |
| Cardiovascular disease     |               |                |               |                |                                        |                                        |
| No                         | 1.00 (reference) | 1.00 (reference) |               |                |                                        |                                        |
| Yes                        | 0.84 (0.38–1.62) | 0.75 (0.36–1.56) |               |                |                                        |                                        |
Only eight (3.7%) patients had serum 25(OH)D levels <5 ng/mL. Due to this small number, model building was not possible using threshold serum 25(OH)D levels of <5 ng/mL. Hence, in this study, only two models were built using threshold serum 25(OH)D levels of ≤30 (M30) and <16 ng/mL (M16), representing cutoffs for vitamin D sufficiency and insufficiency, and vitamin D insufficiency and deficiency, respectively. As this was a retrospective study, information on patients’ diet could not be collected. Future prospective studies can include the collection of dietary information such as consumption of vitamin D-rich foods, which may be an important factor in predicting the risk of vitamin D deficiency. Lastly, the single-center design of this study may limit the generalizability of the results to the entire CKD population in Singapore.

Conclusions
Vitamin D deficiency and insufficiency are common among multiethnic CKD patients in Singapore with an overall prevalence of 82.7%. The risk factors for vitamin D deficiency consistently identified by both models in this study were absence of vitamin D supplementation and type 2 DM; other predictors were non-cancer diagnosis, younger age, Malay race, treatment with calcitriol and higher serum bicarbonate concentrations. The logistic regression models developed in this study can be used to guide healthcare professionals in predicting the likelihood of vitamin D deficiency in CKD patients. The predictors of vitamin D deficiency identified are useful in guiding healthcare professionals on their decisions in vitamin D supplementation to CKD patients who are at risk, before their kidney function deteriorates. However, it is important to note that these identified predictors are only useful for prediction as there may not be a direct cause-and-effect relationship between these predictors and vitamin D deficiency. Due to the small sample size of our study, future prospective studies should be done to validate these findings.

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