25-Hydroxyvitamin D and the risk of incident diabetes in Hong Kong Chinese

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Abstract

Objective: To investigate the relationship between serum 25-hydroxyvitamin D (25(OH)D) and risk of incident diabetes in Hong Kong Chinese, after accounting for the effect of multiple bone- and mineral-related markers.

Design: We conducted a retrospective study on the Hong Kong Osteoporosis Study cohort. Incident diabetes was ascertained using electronic medical records. Serum 25(OH)D was measured at baseline and its association with incident diabetes was evaluated using multivariable Cox proportional-hazard regression.

Participants: Individuals (*n* 4342) aged 20 years or above (1395 men, 2947 women; mean age 54·3 (SD 16·5) years) from the Hong Kong Osteoporosis Study, who were free of diabetes at baseline, were included.

Results: During 40 124·7 person-years of follow-up (a median of 9·2 years), 443 participants developed diabetes. Mean 25(OH)D was 63·34 (SD 13·07) nmol/l. Age-, sex- and BMI-adjusted Cox proportional-hazard regression showed no significant difference in the risk of incident diabetes between the lowest and the highest quintiles of 25(OH)D. In the analysis of the interaction effect between 25(OH)D and serum Ca, the interaction term did not affect the risk of incident diabetes significantly (*P* = 0·694). Similarly, there was no significant interaction of different subgroups (age, sex, BMI, femoral-neck T-score, serum Ca levels) with serum 25(OH)D.

Conclusions: The present study finds that serum vitamin D level is not associated with the risk of incident diabetes in Hong Kong Chinese and this relationship is not modified by serum Ca level.

Vitamin D is a steroid hormone that is important for human health. High prevalence of vitamin D deficiency and insufficiency has become a worldwide health problem[1]. A study in the Hong Kong Chinese population reported that the prevalence of vitamin D deficiency was 43·8 % and that of insufficiency was over 90 %[2]. Vitamin D is well known for its role in bone and mineral metabolism disorders. In addition to its musculoskeletal role, multiple extra-musculoskeletal roles have been proposed. For example, 25-hydroxyvitamin D (25(OH)D), the main circulating form of vitamin D, was shown to be associated with cardio-metabolic risk factors such as hypertension, obesity, glucose intolerance and dyslipidaemia, as well as CVD and

diabetes[3–7]. In animal studies, vitamin D restored insulin tolerance and secretion in vitamin D-deficient diabetic models *in vivo*[8–10]. Human studies have also provided evidence demonstrating that serum 25(OH)D is related to insulin resistance, sensitivity and secretion, and that it has an important role in glucose dysmetabolism[11,12]. Some studies, but not all, have shown that serum 25(OH)D was associated with the risk of diabetes in prospective cohort studies[13–15] Nevertheless, the findings were inconsistent and the role of vitamin D in diabetes is largely unexplored in Chinese[16,17]. We previously showed that serum Ca is a predictor of incident diabetes in the Hong Kong Osteoporosis Study (HKOS)[18], and similar

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findings were also reported in the Atherosclerosis Risk in Communities (ARIC) study[19]; however, whether the association between vitamin D is confounded by serum Ca is unclear. In the present study, we aimed to evaluate the relationship between serum 25(OH)D and the risk of incident diabetes in Hong Kong Chinese, after accounting for the effect of multiple bone- and mineral-related markers.

Methods

Participants

This was a retrospective cohort study using the data from the HKOS. HKOS is a cohort study aiming at studying musculoskeletal and mineral metabolism and their relationship with co-morbidities. Details of the study have been previously described[20,21]. In brief, participants were all Hong Kong residents of self-reported Southern Chinese descent. They were recruited from health fairs and public roadshows and the baseline examinations were carried out between 1995 and 2010. The participants were interviewed, underwent clinical examinations and blood samples were taken after providing written informed consent. Demographic data were collected on anthropometric measurements, socio-economic status, education level, medical and reproductive history using a structured questionnaire by trained interviewers. Lifestyle information including physical activities, smoking and drinking habits was also recorded. Season was defined by the time when the blood samples were taken, as summer/autumn (May to October) and winter/spring (November to April). Physical activities were defined as any regular exercise for more than 1 h/week. Both the protocols of baseline and follow-up study of HKOS have been approved by the Institutional Review Board of the University of Hong Kong and the Hospital Authority Hong Kong West Cluster Hospitals.

In the current study, we included the participants aged 20 years or above (*n* 9202). Those having diabetes at baseline were excluded (*n* 1018). In addition, participants with chronic kidney disease (defined as estimated glomerular filtration rate < 60 ml/min per 1·73 min²; *n* 173), missing variables in the fully adjusted model (*n* 2348), prescriptions of medication affecting mineral metabolism (*n* 122), without serum 25(OH)D measurements (*n* 1185) and with the onset time of diabetes less than 90 d were also excluded (*n* 14). In total, 4342 participants, 1395 males (32·2 %) and 2947 females (67·8 %), were included in final analysis.

Ascertainment of diabetes

The Clinical Data Analysis and Reporting System (CDARS) of the Hong Kong Hospital Authority was used to retrieve electronic medical records for the clinical data collection.

CDARS is run by Hospital Authority, which is a publicly funded statutory body in Hong Kong. Hospital Authority runs forty-three hospitals and 111 general and specialist outpatient clinics, and it provides medical services covering over 90 % of the population of Hong Kong[22]. All the clinical data including patient admission and discharge, diagnosis, laboratory results and prescriptions were recorded in the clinical management system, which are directly transferred to the CDARS. The coding accuracy of CDARS was also validated as over 90 %[23]. We ascertained incident diabetes by one of the following criteria: (i) having a diagnosis of diabetes defined by the *International Classification of Disease, 9th Revision* (ICD-9 code: 250); (ii) having prescription records on medication for diabetes; (iii) having a laboratory record of glycated Hb (HbA1c) > 6·5 % or fasting glucose level > 7 mmol/l; or (iv) having enrolled in a diabetic complication screening programme. We calculated the follow-up length for each participant as the time from the first visit to 1 May 2014, the time of death or diabetes ascertainment, whichever was earliest.

25-Hydroxyvitamin D measurement and other covariates

Serum 25(OH)D was measured by enzymatic immunoassay (EIA; IDS Ltd, UK) at baseline. Minimum detectable level of 25(OH)D was 4.5 ng/ml. Quality controls (QC) were included in each batch of assay and the CV for the assay was less than 10 %. Quality assessment on these EIA-measured 25(OH)D levels using a panel of serum samples with standard 25(OH)D reference values of the CDC Vitamin D Standardization Certification Program (VDSCP) was performed[24,25]. Based on the calculation, the r and r^2 values between the original EIA-measured and standard 25(OH)D values were 0·942 and 0·886, indicating a high correlation between EIA-measured 25(OH)D and standard 25(OH)D. We therefore performed a regression-based correction for the EIA-measured 25(OH)D into QC-harmonized EIA-measured 25(OH)D using the equation: QC-harmonized EIA-measured 25(OH)D = $20·099 + 0·799 \times 25(OH)D_{EIA\ original}$. Serum intact parathyroid hormone was measured by chemiluminometric assay (Chiron Diagnostic Corporation, USA). The inter-assay CV was less than 10 % while the intra-assay CV was less than 11·8 %. Serum Ca, phosphate, albumin and alkaline phosphatase were measured with a Hitachi 747 random access analyser (Roche Molecular Biochemicals, Germany). Bone mineral density (BMD) measurement was performed at the femoral neck using dual-energy X-ray absorptiometry (Hologic QDR 4500, USA); precision of the measurement was 1.5 %. Daily calibration of the equipment and all measurements were performed by professional medical technologists following the manufacturer's instruction. BMD was shown in T-score and we defined the presence of osteoporosis as T-score < −2·5.

Statistical analysis

For descriptive statistics, the baseline characteristics were expressed as mean and standard deviation for continuous variables or as frequency and percentage for categorical variables, and the differences in characteristics between participants with and without incident diabetes were evaluated using the t test and χ^2 test, respectively. The QC-harmonized EIA-measured 25(OH)D levels were used as serum total 25(OH)D in the analysis. The relationship between continuous serum total 25(OH)D level and other variables was assessed by Pearson correlation coefficients. Multivariable Cox proportional-hazard regression models were used to assess the association between 25(OH)D and the risk of incident diabetes. We considered 25(OH)D level as a continuous variable, in quintiles and in vitamin D status categories (<50 nmol/l, <75 nmol/l and ≥75 nmol/l). Variables that were not normally distributed were log-transformed before analysis. Those factors affecting serum 25(OH)D or the risk of diabetes were included in the multivariable Cox regression models. The assumptions of Cox proportional-hazard regression were tested and satisfied. To avoid violating these assumptions, quintiles of serum parathyroid hormone were included in stratum in the models. BMD was shown to be independently associated with diabetes mellitus and serum 25(OH)D, respectively[26,27]. Therefore, we also included femoral-neck BMD T-score in the adjustment models. Model 1 was adjusted for age, sex and BMI. In model 2, there was further adjustment for behavioural and socio-economic variables (smoking, drinking, physical activities and education level) and the biomarkers related to mineral and vitamin D metabolism (serum Ca, serum phosphate, serum alkaline phosphatase, serum parathyroid hormone, femoral-neck BMD T-score, estimated glomerular filtration rate and season). The hazard ratio and 95 % CI of 25(OH)D status and incident diabetes for each quintile were calculated using the highest quintile as reference. Possible interaction of sex, age, BMI, femoral-neck BMD T-score and serum Ca with quintiles of serum 25(OH)D was evaluated by inclusion of the interaction term in the full adjustment model. $P < 0.05$ was defined as statistically significant. All statistical analyses were performed using the statistical software package IBM SPSS Statistics version 21.

Results

Study population

Among 4342 individuals included in the present study, 2947 were female (68 %) with mean age of 52·8 (SD 16·2) years and mean 25(OH)D of 63·34 (SD 13·07) nmol/l. Baseline characteristics of participants who developed or did not develop diabetes are shown in Table 1. Participants with incident diabetes were significantly older, having higher BMI, serum Ca, alkaline phosphatase, 25(OH)D and more physical activities, and with significantly lower serum albumin, phosphate, BMD, education and estimated glomerular filtration rate. In the analysis treating serum 25(OH)D level as a continuous variable, serum 25(OH)D was positively correlated with serum Ca, physical activities and season, and inversely associated with parathyroid hormone, education and estimated glomerular filtration rate. Quintile of serum 25(O)D was significantly associated with education, season, BMD, physical activities, serum total Ca and corrected Ca, and estimated glomerular filtration rate. The associations remained significant after further adjustment for age, sex and BMI (see online supplementary material, Supplemental Table 1).

Follow-up and incident diabetes

During 40 124·7 person-years of follow-up (a median of 9·2 years), 433 participants developed diabetes. The prevalence of incident diabetes observed was 10 % (433 events in 4342 participants), with an estimated incidence rate of 10·8 per 1000 person-years. Number of incident diabetes cases and incidence rate were significantly positively correlated with quintile of 25(OH)D (see online supplementary material, Supplemental Table 1). In Cox proportional-hazard regression, lower quintiles of serum 25(OH)D were not significantly associated with increased risk of incident diabetes when compared with the highest quintile after adjustment for age, sex and BMI in model 1 ($P = 0.59$) and further adjustment for other clinical variables in model 2 ($P = 0.71$; Supplemental Table 2). Interaction analysis in the full adjustment model revealed no significant interaction between quintile of serum 25(OH)D and serum Ca ($P = 0.694$; data not shown). Similarly, there was no significant interaction of different subgroups (age, sex, BMI, femoral-neck T-score, serum Ca levels) with serum 25(OH)D level (Table 2).

Discussion

In the present study, we showed that serum 25(OH)D level was not associated with the risk of incident diabetes in the Hong Kong Chinese population. In addition, an interaction of serum 25(OH)D and serum Ca on the risk of incident of diabetes was not observed.

Level of 25(OH)D was shown to be inversely correlated with parathyroid hormone, smoking and physical activities, the potential confounders of diabetes ($P < 0.001$) as we showed previously (Table 1)[2]. Vitamin D level has been shown to be associated with promotion and development of diabetes by affecting insulin secretion, insulin resistance and pancreatic β-cell dysfunction[11]. Low 25(OH)D level reduced insulin sensitivity and insulin secretion[28,29]. A direct action of 1,25-dihydroxyvitamin D to increase expression of insulin receptor and enhanced insulin sensitivity has been shown[30]. In addition, vitamin D deficiency causes secondary hyperparathyroidism and elevated parathyroid hormone level is associated with insulin synthesis

Table 1 Baseline characteristics (1995–2010), stratified by incident diabetes status at follow-up (censored at 1 May 2014), in Hong Kong Chinese aged 20 years or above (*n* 4342), Hong Kong Osteoporosis Study

Characteristic	Without incident diabetes (*n* 3909)		With incident diabetes (*n* 433)		*P* value	Correlation with serum 25(OH)D†
	Mean or *n*	SD or %	Mean or *n*	SD or %		
Female, *n* and %	2674	68·5	273	63·0	0·023	–
Age (years), mean and SD	51·7	16·3	62·7	10·8	<0·001	–
Education (college/university), *n* and %	1176	30·1	76	17·6	<0·001	−0·089**
Current smoker, *n* and %	240	6·1	27	6·2	0·938	−0·026
Current drinker, *n* and %	369	9·4	43	9·9	0·746	0·022
BMI (kg/m^2), mean and SD	22·45	3·46	25·15	3·89	<0·001	0·014
Physical activities (self-reported > 1 h/week), *n* and %	1963	50·3	248	57·3	0·005	0·127**
Serum Ca (mmol/l), mean and SD	2·388	0·086	2·405	0·081	<0·001	0·117**
Serum albumin-corrected Ca (mmol/l), mean and SD	2·304	0·079	2·327	0·085	<0·001	0·096**
Serum albumin (g/l), mean and SD	44·2	2·7	43·9	3·0	0·038	0·044*
Serum phosphate (mmol/l), mean and SD	1·125	0·150	1·109	0·154	0·034	−0·015
Serum alkaline phosphatase (IU/l), mean and SD	69·18	22·11	77·68	22·36	<0·001	−0·008
Serum parathyroid hormone (pmol/l), mean and SD	3·95	1·52	3·91	1·55	0·694	−0·255**
Femoral-neck BMD T-score, mean and SD	−0·873	1·169	−1·107	1·177	<0·001	0·05**
eGFR (ml/min per 1·73 min^2), mean and SD	101·91	21·40	94·16	18·58	<0·001	−0·110**
Season (summer/autumn), *n* and %	2291	58·7	250	57·7	0·727	0·124**
Serum 25(OH)D (nmol/l), mean and SD	63·08	13·00	65·73	13·41	<0·001	–

BMD, bone mineral density; eGFR, estimated glomerular filtration rate; 25(OH)D, 25-hydroxyvitamin D.
*$P < 0.05$, **$P < 0.001$.
†Pearson correlation coefficient adjusted for age and sex.

Table 2 Hazard ratios (HR) of serum 25-hydroxyvitamin D (25(OH)D) level (continuous) with incident diabetes from baseline (1995–2010) to follow-up (censored at 1 May 2014), according to subgroups, in Hong Kong Chinese aged 20 years or above (*n* 4342), Hong Kong Osteoporosis Study

	Model 1		Model 2		*P* for interaction†
	HR	95 % CI	HR	95 % CI	
Age (years)					
≤60	0·99	0·98, 1·00	0·99	0·98, 1·00	0·103
>60	1·00	0·99, 1·01	1·00	0·99, 1·01	
Sex					
Male	1·00	0·99, 1·01	1·00	0·98, 1·01	0·802
Female	0·99	0·99, 1·01	1·00	0·99, 1·01	
BMI (kg/m^2)					
<24	1·00	0·99, 1·01	1·00	0·99, 1·01	0·564
24–28	0·99	0·98, 1·00	0·99	0·98, 1·00	
>28	1·01	0·99, 1·02	1·01	0·99, 1·02	
Femoral-neck BMD T-score					
≤−2·5	1·00	0·98, 1·02	1·00	0·99, 1·01	0·658
>−2·5 to −1·0	1·00	0·99, 1·01	1·00	0·98, 1·01	
>−1·0	1·00	0·99, 1·01	1·00	0·99, 1·01	
Serum Ca level (mmol/l)					
<2·33	1·00	0·99, 1·02	0·99	0·97, 1·02	0·763
2·33–2·38	0·99	0·98, 1·01	0·99	0·98, 1·01	
2·39–2·44	0·99	0·98, 1·01	0·97	0·98, 1·00	
>2·44	1·00	0·99, 1·01	1·00	0·99, 1·01	

BMD, bone mineral density.
Model 1: adjusted for age, sex and BMI.
Model 2: model 1 further adjusted for lifestyle factors (smoking, drinking, physical activities, education levels), biomarkers of vitamin D (season, estimated glomerular filtration rate, serum Ca, serum albumin, serum phosphate, serum alkaline phosphatase, serum parathyroid hormone and femoral-neck T-score).
†*P* value of the interaction term of the subgroups with 25(OH)D level in model 2.

and secretion as well as related to impaired glycaemic control[12,31]. Some studies also demonstrated that vitamin D supplementation improved HbA1c in diabetes mellitus patients with higher baseline HbA1c or with severe vitamin D deficiency[32,33]. All these evidences supported the link between those primary biomarkers characterizing diabetes and vitamin D level. However, we did not have complete information across the whole population on homeostatic model assessment of insulin resistance, fasting glucose or oral glucose tolerance test results, so we are not able to study any unfavourable changes in these biomarkers of glucose homeostasis with serum 25(OH)D level.

25(OH)D level was significantly higher in those participants having incident diabetes than in those without diabetes (63·08 (SD 13·00) v. 65·73 (SD 13·41) nmol/l) in the current study (Table 1) and we demonstrated a significant positive correlation between quintiles of 25(OH)D and incidence rate of diabetes (Supplementary Table 1). Although an increased relative risk was reported in previous meta-analyses[34,35], we did not find any statistically significant increased risk of incident diabetes in our study population. A similar negative result was observed if we stratified 25(OH)D level according to the suggested threshold levels of vitamin D status. These may suggest that 25(OH)D itself was not associated with the risk of incident diabetes, but as a biomarker it correlated with the predictors positively associated with incident diabetes. Our results are consistent with some previous studies[36–38]. Negative findings were also found in two other nested case–control studies, one in Finland[14] and the other one being the Women's Health Initiative Study[39], in which only females or older females were studied. In randomized controlled trials and meta-analysis, no association was observed between vitamin D supplementation and glucose homeostasis[40–42].

On the other hand, our results stand in contrast to many published epidemiology studies and several meta-analyses. They showed a correlation between low vitamin D and the higher risk of diabetes. However, some studies were based on self-reported supplementary or dietary vitamin D intake without measurement of serum 25(OH)D level[13,43,44]. A large prospective population-based study of 7791 individuals demonstrated a significant inverse association with risk in older men but not in older women[45]. Several recent meta-analyses on cross-sectional studies and prospective studies also demonstrated that low 25(OH)D level was correlated with higher risk of type 2 diabetes[34,35,39,45]. However, these analyses were performed mainly on older age groups and those significant associations were diminished after adjustment for other confounders such as BMI[38].

Discrepancy of the findings may be explained by the difference in consideration of confounders in different studies, such as age, sex, BMI and the ascertainment of diabetes. In the current study, we included serum Ca level as another potential confounder in the relationship between vitamin D and risk of diabetes. It is well known that vitamin D relates with bone metabolism and 25(OH)D is positively associated with Ca level. Moreover, our previous study showed that Ca was a predictor for incidence of diabetes and that higher serum Ca level was significantly associated with higher risk of incident diabetes among participants from the same population[18]; such relationship was further supported by the ARIC study subsequently[19]. As an evaluation of this hypothesized interaction effect of vitamin D with Ca homeostasis on incident diabetes, we introduced an interaction term in the Cox regression model. However, we found that there was no interaction between

quintiles of 25(OH)D and serum Ca level, suggesting that the null association observed with 25(OH)D was not confounded by the effect of Ca. In stepwise Cox regression on the association of 25(OH)D with incident diabetes with only age, sex, BMI and serum Ca as covariates, adjustment for age and sex only already attenuated the associations substantially, making quintile of 25(OH)D become statistically non-significant (see online supplementary material, Supplemental Table 3).

Furthermore, we also evaluated the interaction between serum 25(OH)D level and age (older or younger than 60 years), sex, BMI, femoral-neck T-score and quantile of serum Ca level on the risk of incident diabetes; however, null associations were observed (Table 2). Compared with other studies showing significant associations in older people and in males, there was no such association in either sex and age group in our study population. However, we demonstrated that age group (younger or older than 60 years) may have a possible interaction with quintile of serum 25(OH)D on the hazard ratios with a marginally significant P for interaction value ($P = 0·051$; data not shown). This may echo our previous finding in Hong Kong Chinese on the significant difference between these age groups in the relationship of 25(OH)D with parathyroid hormone, which was shown to be an independent predictor for insulin secretion and sensitivity[2,12].

Different methods for the ascertainment of diabetes were used in different studies. Some studies depended only on self-reported diabetes[46,47] or the diabetic medication records in a registry[14,39] without diagnosing diabetes with HbA1c, fasting glucose or oral glucose tolerance test data. This may introduce misclassification of diagnosis and exclude those with undiagnosed diabetes, because it is well known that type 2 diabetes mellitus is under-diagnosed in the general population. In the current study, we validated the diabetes ascertainment by telephone interview with 10 % of the participants and demonstrated that self-reported diabetes and diabetes ascertainment from electronic medical records were highly correlated (κ statistic = 0·881)[18].

The inconsistent results from observational studies suggested that randomized interventional trials and genetic epidemiological studies might help to validate the role of vitamin D in diabetes in human studies. However, in more recent randomized controlled trials and meta-analyses on vitamin D supplementation, vitamin D supplement did not improve glycaemic control and prevent diabetes[41,48–50]. Further studies are needed to study any causal relationship between vitamin D and diabetes and to confirm those observational positive findings, before recommendation of supplemental vitamin D as a preventive measurement for diabetes in the healthy general population.

There are several strengths in the present study. First, the participants were from the Hong Kong Chinese general population of both sexes and from age 20 years upwards. Second, the present study is the first to consider the interaction with the potential predictor, Ca, on the protective

effect of vitamin D on the development of diabetes. We included the potential confounder, serum Ca level, in the analysis of the association between serum 25(OH)D level and risk of incidence diabetes. There are nevertheless several limitations in our study. First, we did not have complete data for the whole study population on glucose homeostasis, such as fasting glucose, oral glucose tolerance test and homeostatic model assessment of insulin resistance results, so we did not study the relationship between glucose homeostasis and vitamin D. Second, there were no data on vitamin D supplementation. Third, we did not measure 25(OH)D level using the gold-standard LC–MS method. However, the methodology used in our study should give results that are in high correlation with results from LC–MS ($R > 0.93$)[51].

Conclusion

In conclusion, serum vitamin D level was not associated with the risk of incident diabetes in Hong Kong Chinese and did not interact with serum Ca in the relationship. The positive correlation between serum 25(OH)D and incidence rate of diabetes may suggest serum vitamin D as a biomarker for the possible predictors of incident diabetes. We recommend caution in evaluating the effect of serum vitamin D on risk of diabetes without considering serum Ca levels.

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Supplementary material

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