Influence of vitamin D binding protein polymorphism, demographics and lifestyle factors on vitamin D status in healthy Malaysian pregnant women

Siew-Siew Lee
Universiti Putra Malaysia https://orcid.org/0000-0001-6505-8615
King-Hwa Ling
Universiti Putra Malaysia
Maiza Tusimin
Prince Court Medical Centre Kuala Lumpur
Raman Subramaniam
Fetal Medicine and Gynecology Centre
Kartini Farah Rahim
Avisena Specialist Hospital
Su-Peng Loh (sploh@upm.edu.my)
https://orcid.org/0000-0002-5609-8065

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Abstract

Background

Vitamin D deficiency (VDD) has been related to vitamin D binding protein (*GC*) gene polymorphism, demographics and lifestyle factors in different populations. However, previous studies examining the factors associated with VDD during pregnancy were restricted to the study of only demographics and lifestyle factors or genetic factors alone. Thus, this study assesses the associations of *GC* gene polymorphism, environmental and lifestyle factors with VDD in Malaysian pregnant women.

Method:

Information on demographics, dietary vitamin D intake from supplement and food, time spent outdoors, skin type and clothing were collected by questionnaire. Plasma total 25-hydroxyvitamin D (25OHD) levels were measured using Ultra-High-Performance Chromatography (UHPLC). Maternal *GC* single nucleotide polymorphisms (SNPs) (rs4588 and rs7041) were determined using restriction fragment length polymorphism (RFLP).

Results

Results showed that 50.2% of pregnant women were vitamin D deficient (25OHD < 30 nmol/L). VDD (25OHD < 30 nmol/L) were significantly associated with age, veiled clothing, maternal vitamin D intakes, both from food and supplement, and *GC* rs7041(and *GC* diplotypes). In contrast to previous studies in non-pregnant population, this study found that CC genotype for SNP *GC* rs7041, *GC* 1 s-1 s and *GC* If-2 were significantly associated with increased risk of VDD (25OHD < 30 nmol/L).

Conclusions

The high prevalence of maternal VDD reported in the present study suggests the need for urgent development and implementation of vitamin D supplementation or fortification strategies to reduce VDD among pregnant women. The discrepancy in the association of *GC* rs7041 with VDD reflects the differential in the factors associated with vitamin D deficiency in pregnancy compared to non-pregnant state

Background

Vitamin D is well known for its role in regulating bone metabolism by stimulating intestinal calcium and phosphorus absorption [1]. During pregnancy, apart from its role in bone metabolism, recent evidence has shown that vitamin D also plays a vital role in cellular proliferation and regulation, trophoblast invasion, and immunomodulation at the maternal-fetal interface [2, 3]. Maternal vitamin D deficiency (VDD) has

been suggested as a potential mechanism mediating the pathogenesis of adverse pregnancy and neonatal outcomes, including preeclampsia [4, 5], gestational diabetes [6], small for gestational age [7, 8] and preterm birth [9].

Globally, it is estimated that 50–80% of pregnant women worldwide are vitamin D deficient (25-hydroxyvitamin D (25OHD) < 50 nmol/L) [10]. Findings from the previous epidemiological studies have demonstrated that VDD was high in countries located at a high latitude [10]. Recent studies also reported a high prevalence of VDD in countries with abundant sunshine such as Spain [11], India [12], Thailand [13, 14], and Malaysia [15]. In countries at high latitudes, such as the United Kingdom [16] and the Netherland [17], there is routine vitamin D supplementation recommendation for a specific group at risk, particularly pregnant women. Despite the reported high prevalence of VDD in pregnant women in the region with abundant sunshine like Malaysia, routine vitamin D supplementation recommendation for pregnant women has not yet been established. This is owing to insufficient data to inform the development of vitamin D supplementation recommendations for pregnant women.

Previously, VDD has been related to environmental and lifestyle factors, including latitude, season, sun exposure, skin type, clothing, dietary vitamin D intake, Body Mass Index (BMI) and ethnicity [18]. Several genome-wide association studies (GWAS) and candidate gene studies have shown that single nucleotide polymorphisms (SNPs) located in or near the genes that encode the key enzymes or protein for the metabolism, transportation and action of the mechanism of vitamin D, such as cytochrome P450-2R1 (*CYP2R1)*, cytochrome P450-27B1 (*CYP27B1)*, and vitamin D binding protein (*GC)* genes, were associated with vitamin D status [19–22]. Nonetheless, the studies reporting the association of these SNPs and vitamin D status were largely conducted in the non-pregnant population [19–22]. Also, previous studies examining the factors associated with VDD during pregnancy were restricted to the study of only environmental [15, 23, 24] or genetic factors alone [25–28].

In a review by DA Jolliffe, RT Walton, CJ Griffiths and AR Martineau [29] that included a total of 120 genetic associations studies, they found that SNPs in *GC*, particularly rs7041 and rs4588, are the most reported SNPs associated with the level of 25OHD. Furthermore, SNP rs7041 and rs4588 are missense SNPs (functional SNPs), which the variation in the nucleotide has resulted in the change in amino acid and vitamin D binding protein glycosylation pattern. For rs7041, A to C allele transversion causes the change of amino acid aspartic acid (CT**A**) to glutamic acid (CT**C**), whereas for rs4588, a G to T allele and glycosylation patterns of the vitamin D binding protein [30]. The combination of the two SNPs (rs4588 and rs7041) has resulted in three different *GC* haplotypes/isoforms: *GC*1f (A allele rs7041 and G allele rs4588), *GC*1s (C allele rs7041 and G allele rs4588) and *GC*2 (A allele rs7041 and T allele rs4588). These three *GC* haplotype/isoforms give rise to 6 *GC* diplotypes: 1f/1f, 1 s/1 s, 2/2, 1f/1 s, 1f/2, 1 s/2. During pregnancy, the concentration of vitamin D binding protein is elevated two to three folds higher compared to the non-pregnant state [31]. Thus, the effect of *GC* SNPs on vitamin D status may be different from those reported in the non-pregnant population. Therefore, this study aims to assess 1) the prevalence of VDD, and 2) the associations of both environmental, lifestyle factors and *GC* gene polymorphism (rs7041, rs4588 and the *GC* diplotypes) with VDD among healthy Malaysian pregnant women.

Methods

Study design

This study was conducted between October 2015 through February 2017. A total of 217 healthy pregnant women were conveniently recruited while admitting for delivery at the Department of Gynaecology and Obstetrics Hospital Serdang, Selangor, Malaysia. Inclusion criteria were Malaysian, aged 19 to 40 years, singleton pregnancy and week of pregnancy of ≥ 37 weeks. Pregnant women who were diagnosed with pre-existing systemic disease, pregnancy complications, and had a history of bone and renal disorders, were excluded from the study. Ethical approval was obtained from the Medical Research and Ethics Committee Ministry of Health Malaysia (MREC) with the ID: NMRR-15-786-24865.

Data collection and blood sampling

Information on maternal ethnicity, education level, employment status and household income were self-reported using an interviewer-administered questionnaire. Maternal age, gestational age, last menstrual period (LMP), first booking (date, weeks, and ultrasound scan), gravidity, pre-pregnancy weight and heights were obtained from the electronic medical record and antenatal record. Gestational age was determined by LMP and confirmed by the first dating scan. Body Mass Index (BMI) was calculated as body weight divided by squared body height (kg/m^2). Venous blood was collected from pregnant women on the day of labour. All collected blood samples were processed within the days of collection: after centrifugation, plasma was aliquoted and buffy coat was collected for subsequent deoxyribonucleic acid (DNA) extraction.

The duration of exposure to sunlight per week was estimated using a questionnaire adapted from LM Hall, MG Kimlin, PA Aronov, BD Hammock, JR Slusser, LR Woodhouse and CB Stephensen [32]. Pregnant women were asked about their outdoor activities from 7 am-7 pm during weekdays and weekends. Information on the type of activity, duration (in minutes), usual outdoor attire, frequency (per week), use of glove, umbrella, and sunscreen were recorded. Based on the attire worn, the percent of body surface area (BSA) exposed to sunlight was estimated using "Rule of Nine".

To estimate the skin colour of the study participants, the Fitzpatrick scale [33] was used. Based on the women skin colour and skin tanning evaluation, study participants were classified into six different skin phototypes. In this study, as the sample size in the subset of each skin type, particularly skin type I, II, V and VI, was small, skin colour types were dichotomized into light skin colour (Fitzpatrick scale I to III) vs. dark skin colour (Fitzpatrick scale IV and V) [34].

Daily vitamin D intake from dietary and supplemental sources, was assessed using a vitamin D-specific semi-quantitative Food Frequency Questionnaire (FFQ), adapted from a previous study [35]. Pregnant women were asked to recall the brand (for commercial food), frequency and serving size of the listed food they had consumed over the past one month. At the same time, pregnant women also provided the

information regarding their supplemental intakes over the past one month: supplements (e.g. brand name, type of supplements, and specific nutrient), frequency and dosage of intake.

Measurement of plasma 25OHD

Plasma concentrations of $25OHD_3$ and $25OHD_2$ were determined by ultra-high-performance chromatography (UHPLC) and summed to give total plasma 25OHD. The inter-assay coefficient of variation at 50 nmol/L were 6% and 7% for $25OHD_3$ and $25OHD_2$, respectively. Multiple cutoffs (< 25, < 30 and < 50 nmol/L) were used to describe the prevalence of maternal VDD. The cutoff of IOM (< 30 nmol/L), the cut-off associated with an increased risk of VDD [36], was used in the analysis of the factors associated with maternal VDD.

Genotyping

DNA was extracted from the buffy coat using the QIAamp DNA blood kit (QIAGEN, Germany) according to the manufacturer's protocol. DNA yields and quality were determined using NanoVue Plus UV spectrophotometer (GE Healthcare, USA).

Genotyping of *GC* SNPs rs4588 and rs7041 were carried out by restriction fragment length polymorphism (RFLP). PCR was performed in a thermocycler (Thermo Fisher Scientific, USA). The PCR reaction was prepared in a total volume of 20 μL: 10 μL of 2X GoTaq® G2 Green Master Mix (Promega, USA), 1 μL each of 10 nmol forward primer (5'-AAATAATGAGCAAATGAAAGAAGAC-3') and reverse primer (5'-CAATAACAGCAAAGAAATGAGTAGA-3'), 3 μL of nuclease-free water and 5 μL of DNA (15 ng/μL). The PCR conditions for amplification included an initial step of denaturation at 95 °C for 10 minutes followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at 51 °C for 45 s and elongation at 72 °C for 45 s, and finally a step of final extension step at 72 °C for 7 min. The PCR product, a 483-bp fragment, was then digested separately by *HaeIII* (for rs7041) and *StyI* (for rs 4588) (New England Biolabs Inc., USA) in a total reaction volume of 20 μL. The digested products were checked by electrophoresis on 1.5% agarose gel stained with ethidium bromide.

Statistical analysis

All statistical analysis was performed using SPSS version 21.0 (SPSS Inc., Chicago, USA). All the continuous variables were assessed for normality using skewness and kurtosis test. Mean and standard deviation (SD) were presented for normally distributed variables, whereas median and interquartile range (IQR) were presented for skewed variables. All the categorical variables were reported as numbers and proportions. Differences in the proportion of *GC* SNPs and diplotypes between ethnicity and the Hardy-Weinberg Equilibrium (HWE) for each SNPs were examined using Chi-square test.

Individual associations between factors and VDD (25OHD < 30 nmol/L) were determined using univariate binary logistic regression. To further evaluate the joint associations of demographic, lifestyle and genetic factors with the risk of maternal VDD, multivariate logistic regression was performed. Variables that had p-value < 0.25 in univariate analyses and biologically important were included in multivariate analyses. Cramer's V and Pearson's correlation coefficient were used to test the associations between variable.

Variables with $r^2 > 0.6$ were considered highly correlated and excluded from the multivariate regression. Two separate multivariate models were constructed to determine the factors associated with VDD. Model 1 included the selected demographics, lifestyle factors as well as the two *GC* SNPs (rs7041 and rs4588); Model 2 consisted of the selected demographics and lifestyle factors as well as *GC* diplotypes. All the statistical significance was set at p-value < 0.05.

Results

Characteristic of participants

Table 1 summarises the characteristics of the study participants. The majority of the study participants (86.2%) were Malays with the mean age of 29 ± 4 years. The proportion of participants with an education level up to tertiary education was slightly higher (56.7%) compared with secondary or lower (43.3%). Nearly half of the women had a monthly household income at the range of RM3001 to RM5000 (approximately USD 750–1600). One-hundred and forty-one participants (65.0%) were enrolled between April and November, while 76 (35.0%) were enrolled between December and March.

The median time spent outdoors per week was 1.9 (range: 0.7–4.1) hour. As the majority of the study participants were of Malay, it was observed that as many as 82.5% of participants were veiled. Median % BSA exposed to sunlight was 6.5%, which was equivalent to veiled clothing (covered on the head, with long sleeve and long pants) and sandals. Not more than half of the respondents (40.1%) were taking vitamin D containing supplements.

Table 2 shows the distributions of the two *GC* SNPs and diplotypes in the study participants. The genotype distributions for the two SNPs were in Hardy-Weinberg equilibrium ($\chi^2 < 0.3841$, $p > 0.05$). Minor allele frequencies for *GC* rs4588 (T allele) and *GC* rs7041 (C allele) were 21% and 37%, respectively. The results showed heterogeneity of the allele frequencies for the two *GC* SNPs between ethnicity. The frequency of the rs4588 T allele was significantly higher in Chinese (35.0%) than Malay, Indians and other ethnicities (19.0%) ($\chi^2 = 4.904$, $p < 0.015$). The *GC* rs7041 C allele showed the trend of lower frequency in Chinese (23.0%) than non-Chinese (38.0%), although the difference was not statistically significant ($p = 0.051$). For *GC* diplotypes, 1f-1 s was the most frequent diplotypes in pregnant women but diplotypes 2–2 had a very low frequency (2.8%) in the study population.

Table 1
Characteristics of study participants

Characteristics	N = 217
Ethnicity, n (%)	
Malay	187 (86.2)
Chinese	20 (9.2)
Indians and others	10 (4.6)
Parity, n (%)	
Nulliparous	58 (26.7)
Multiparous	159 (73.3)
Maternal highest education level, n (%)	
Secondary and lower	94 (43.3)
Tertiary and higher	123 (56.7)
Household Income (per month), n (%)	
≤ RM3000	64 (30.0)
RM3001-RM5000	94 (44.1)
≥ RM5001	55 (25.9)
Month of sampling, n (%)	
December-March	76 (35.0)
April-November	141 (65.0)
Fitzpatrick skin type, n (%)	
Light (Type I, II, III)	97 (44.7)
Dark (Type IV, V &VI)	120 (55.3)
Veiled, n (%)	
Yes	179 (82.5)
No	38 (17.5)
Use of vitamin D supplement, n (%)	
Yes	87 (40.1)
No	130 (59.9)

Characteristics	N = 217
Maternal age (in years) *	29 ± 4
Week of pregnancy*	39.1 ± 1.1
Pre-pregnancy BMI (kg/m2) *	23.7 ± 5.1
Time spent outdoors per week (hours)#	1.9 (0.7–4.1)
% BSA exposed to sunlight#	6.5 (6.5–6.5)
VD intake from (µg/day) *	8.3 ± 5.0
VD intake from supplement (µg/day) *	3.8 ± 5.6
Plasma Total 25OHD (nmol/L) #	29.8(18.8–43.5)

Note

Categorical variables are presented as n (percentages); * variable is presented as mean ± standard deviation; # variables are presented as median (first quartile, third quartile)

Table 2
Distribution of *GC* genotypes and diplotypes by ethnicity

		Total (n = 217)	Malay, Indians & others (n = 197)	Chinese (n = 20)	χ^2	p-value
GC **rs4588**						
Allele	G	(79.5)	(81.0)	(65.0)	4.909	0.015
	T	(20.5)	(19.0)	(35.0)		
Genotype	GG	135 (62.2)	127 (65.5)	8 (40.0)		
	GT	76 (35.0)	66 (33.5)	10 (50.0)		
	TT	6 (2.8)	4 (2.0)	2 (10.0)		
GC **rs7041**						
Allele	A	(63.4)	(62.0)	(77.0)	3.793	0.051
	C	(36.6)	(38.0)	(23.0)		
Genotype	AA	89 (41.0)	78 (39.6)	11 (55.0)		
	AC	97 (44.7)	88 (44.7)	9 (45.0)		
	CC	31 (14.3)	31 (15.7)	0		
GC **diplotypes (rs4588 + rs7041)**						
Allele	1f	(43.1)	(43.1)	(42.5)	6.934	0.031
	1 s	(36.6)	(38.1)	(22.5)		
	2	(20.3)	(18.8)	(35.0)		
Diplotypes	1f-1f	44 (20.3)	40 (20.3)	4 (20.0)		
	1 s-1 s	31 (14.3)	31 (15.7)	0 (0.0)		
	1f-1 s	60 (27.6)	56 (28.4)	4 (20.0)		
	1f-2	39 (18.0)	34 (17.3)	5 (25.0)		
	1 s-2	37 (17.1)	32 (16.2)	5 (25.0)		
	2–2	6 (2.8)	4 (2.0)	2 (10.0)		
Categorical variables are presented as n (percentages)						

Prevalence of VDD

The median (IQR) total 25OHD concentration was 29.8 nmol/L (Q_1-Q_3 = 18.8–43.5 nmol/L); as many as 41.9% of pregnant women had 25OHD < 25 nmol/L, 50.2% were < 30 nmol/L and 82.2% were < 50 nmol/L

(Fig. 1).

Factors associated with maternal vitamin D deficiency

logistic regression analyses showed that the risk factors for VDD (25OHD < 30 nmol/L) were higher maternal age and veiled ($p < 0.05$) (Table 3). In contrast, vitamin D intake from food, supplements and the %BSA were the protective factors ($p < 0.05$).

For genetic factors, the results revealed that the homozygous mutant (CC genotype) of *GC* rs7041 was associated with an increased risk of VDD when compared to other genotypes (Table 4). Nonetheless, no significant association was found between VDD with SNP *GC* rs4588. *GC* diplotype (combinations of rs7041 and rs4588) 1 s-1 s and 1f-2 were associated with an increased risk of VDD.

Table 3
Associations between demographic and lifestyle factors with maternal VDD (25OHD < 30 nmol/L) in univariate analysis

Covariates	Crude OR	95% CI	p-value
Ethnicity			
Malay, Indians and others	2.56	0.94–6.93	0.065
Chinese	1.00	Reference	
Parity			
Nulliparous	0.90	0.49, 1.64	0.728
Multiparous	1.00	Reference	
Maternal highest education level			
Secondary and lower	0.98	0.58–1.68	0.953
Tertiary and higher	1.00	Reference	
Household Income (per month)			
≤ RM3000	0.72	0.35–1.49	0.373
RM3001-RM5000	0.63	0.32–1.24	0.181
≥ RM5001	1.00	Reference	
Month of sampling			
December-March	0.66	0.37–1.15	0.142
April-November	1.00	Reference	
Fitzpatrick skin type			
Light (Type I, II, III)	1.00	Reference	
Dark (Type IV, V &VI)	0.63	0.36–1.07	0.087
Veiled			
Yes	2.56	1.22–5.40	0.013
No	1.00	Reference	
Maternal age	1.08	1.01–1.16	0.018
Week of pregnancy	0.93	0.74–1.18	0.572
Pre-pregnancy BMI	1.02	0.97–1.08	0.441

BMI, body mass index; VD, vitamin D

Covariates	Crude OR	95% CI	p-value
Time spent outdoors per week (h)	0.68	0.35–1.33	0.262
BSA exposed to sunlight	0.32	0.11–0.95	0.039
VD intake from food	0.93	0.88–0.99	0.014
VD intake from Supplements	0.93	0.89–0.98	0.008
BMI, body mass index; VD, vitamin D			

Table 4
Associations between *GC* genotypes and diplotypes with maternal VDD (25OHD < 30 nmol/L) in univariate analysis

		Crude OR	95% CI	p-value
GC rs4588	GG	1.00	Reference	
	GT + TT	1.07	0.61–1.87	0.814
GC rs7041	AA	0.65	0.37–1.17	0.654
	AC	1.00	Reference	
	CC	2.87	1.22–6.74	0.016
GC diplotype	1f-1f	1.00	Reference	-
	1 s-1 s	2.76	1.06–7.22	0.038
	1f-1 s	1.07	0.49–2.36	0.854
	1 s-2	0.80	0.33–1.96	0.626
	1f-2	2.40	0.97–5.70	0.059
	2–2	1.30	0.24–7.26	0.753

To further assess the joint effects of demographic, lifestyle and *GC* SNPs on the risk of VDD, multivariate logistic regression was performed. Variables that had p-value < 0.25 in univariate analyses and biologically important were included in multivariate analyses. Variables that fulfilling these criteria are maternal age, ethnicity, household income (RM3001-RM5000), month of sampling, vitamin D intake from food, supplements, Fitzpatrick skin type, BSA exposed to sunlight, veiled, SNP rs7041, SNP rs4588 and *GC* diplotypes.

Variables that were strongly correlated were excluded to avoid the possibility of a multicollinearity problem. As % BSA exposed to sun and veiled were strongly correlated, a decision was made to include only veiled in the multivariate analysis. Given that ethnicity was overlapped with the variables veiled and skin colour, ethnicity was not included in the multivariate analyses. Based on univariate analysis, *GC*

rs7041 appeared to have a recessive effect on vitamin D status, while rs4588 appeared to have a dominant effect. Thus, the recessive and dominant models were accounted for SNP rs7041 and rs4588, respectively in the multivariate models. Therefore, the final multivariate models included: age, household income (RM3001-RM5000), the month of sampling, vitamin D intake from food, vitamin D intake from supplements, Fitzpatrick skin type, veiled, *GC* rs704 and rs4588 genotype (or diplotypes).

In the backward stepwise multivariate analysis (model 1; Table 5), maternal age, vitamin D intake from food, vitamin D intake from supplements, veiled, *GC* rs7041 SNP and month of blood sampling was significantly associated with VDD (Table 5). The results demonstrated that the risk of VDD increased as the age increased but decreased as the vitamin D intake from food and supplements increased. Pregnant women who veiled had about 4 times higher risk of VDD compared to pregnant women who did not veil. Likewise, pregnant women who carried homozygous mutant in *GC* rs7041 SNP had about 3 times higher risk to have VDD compared to other women who carried other genotypes. In a separate multivariate model (model 2), the analysis showed that pregnant women who had diplotypes 1f-2 and 1 s-1 s both had 3–4 times higher risk of VDD compared to pregnant women of other diplotypes. Time spent outdoor, skin type and other factors were not significantly associated with maternal vitamin D status.

Table 5
Multivariate models of demographic, lifestyle, *GC* genotypes and diplotypes with VDD (25OHD < 30 nmol/L)

Variables	Model 1			Model 2		
	aOR	95% CI	p-value	aOR	95% CI	p-value
Age	1.11	1.03, 1.19	0.006	1.11	1.03, 1.20	0.005
Dietary VD intake	0.89	0.84, 0.96	0.001	0.89	0.83, 0.95	< 0.001
Supplemental VD intake	0.92	0.87, 0.98	0.004	0.91	0.86, 0.97	0.003
Veiled (Yes)	4.27	1.80, 10.11	0.001	4.65	1.92, 11.25	0.001
Month of sampling (Dec-Mar)	0.50	0.26, 0.94	0.030	0.52	0.28, 0.99	0.047
GC rs7041	2.96	1.13, 7.76	0.028	-	-	-
GC rs4588	0.56	0.29, 1.08	0.084	-	-	-
GC Diplotype 1f-2	-	-	-	3.75	1.60, 8.77	0.002
GC Diplotye 1 s-1 s	-	-	-	3.08	1.19, 7.99	0.021

Discussion

Malaysia is a tropical country where sunlight is available throughout the year. However, we have shown that the prevalence of VDD among pregnant women is high. The estimate of 82.0% of maternal plasma 25OHD < 50 nmol/L was consistent with the studies conducted in the local urban area (Kuala Lumpur).

The studies reported 90% and 72% VDD (25OHD < 50 nmol/L) among pregnant women at first trimester [15] and delivery [24], respectively. However, the prevalence reported in the present study was higher compared to our previous study conducted among pregnant women from a private hospital in Kuala Lumpur [37]. The variation could be attributed to differences in the proportion of ethnicity and socio-economic background among study participants in present and previous studies. The prevalence of maternal deficiency reported in the present study was comparable to the prevalence reported in countries at high latitude, which included Ireland (80.0%) [38], Germany (77.0%) [39], China (75.0%) [40] and Japan (73.0%) [41]. Nonetheless, the prevalence of maternal deficiency reported in the present study is higher compared to those reported in Thailand (20–40%) [13, 14] and India (66.0%) [12] among pregnant women at delivery and third trimester.

In previous studies, the factors that had been reported to be significantly associated with maternal vitamin D status varied from study to study. These discrepancies suggest that vitamin D status is country-, ethnic- or subgroup-specific. Besides different geographically (latitude and UV availability), each country implements its dietary recommendation, vitamin D food fortification strategies and supplementation policy. Likewise, each subgroup or ethnic groups has a different eating habit, clothing, skin colour, physical activity, attitude towards sun exposure and genetic makeup. A combination and interaction of these factors may put a population at risk of VDD. From another context, to address the needs and inform the potential intervention, the novel factors associated with increased risk of VDD in a specific country, ethnic, or sub-group should be explored. In this study, we comprehensively assessed the demographics and lifestyle factors as well as *GC* polymorphism that associated with VDD in our study population.

Despite abundance in sunlight throughout the year, we found no association between time spent outdoors and maternal VDD. Likewise, skin type was not associated with the risk of maternal VDD. These null results are consistent with previous local studies, which examined factors associated with VDD in early pregnancy [15]. They found no association between Fitzpatrick classification, melanin indices, sun protection score and sunlight exposure with maternal VDD [15]. The null results may be driven by a high proportion of Malay ethnic group in the study, with a majority of them (82.5%) were veiled. In support of this, veiled was identified as a risk of VDD, which veiled women were about 4 times higher risk than unveiled women. This finding is expected as UVB does not transmit through clothing. This finding is in agreement with a study conducted in Saudi Arabia [42], which the investigators reported that veiled significantly associated with increased risk of VDD.

The positive association between vitamin D intake from food and supplements and maternal VDD is in agreement with some [34, 41, 43–48], but not all [39] of the previous studies. It appears that vitamin D intake from food and supplements contributed significantly to 25OHD level in population, in which sun exposure is minimum or dermal synthesis of 25OHD is limited. For instances, recent studies from two high latitude countries, Sweden [49] and Switzerland [34, 47] reported that supplements use, but not time spent outdoors was associated with decreased risk of VDD. Likewise, in a large Chinese study, Yun and colleagues [40] demonstrated that vitamin D supplements use was associated with vitamin D status

during winter when the sun exposure is limited but not significantly associated during autumn. Our study produced results that corroborate with their findings, in which sun exposure did not contribute to the risk of VDD, but dietary vitamin D intake did.

Previous studies investigating the association of *GC* rs7041 with 25OHD had demonstrated C allele associated with decreased risk of VDD in pregnant [25–27, 50] and non-pregnant population [19–22]. In contrast to the previous studies, the C allele was found to increase the risk of VDD in pregnant women in the current study. The discrepancy could be due to the factors included in the present studies were different from previous findings. For instance, the previous studies that examined the factors associated with maternal VDD have been restricted to the only environmental [15, 23, 24] or genetic variables alone [25–28]. Additionally, it is possible that changes in the metabolism of vitamin D, notably elevation of circulation vitamin D binding protein concentration, may change the association of *GC* SNP with vitamin D status. The discrepancy suggests that the tremendous change in the metabolism of vitamin D during pregnancy may cause a differential in the factors associated with VDD in pregnancy compared to the non-pregnant state.

This study has the limitation as the data was collected in a public hospital in which the study finding may not be generalised to the pregnant women who attend the private Hospital. However, this study assessed a wide range of possible factors associated with maternal VDD: vitamin D intake from diet and supplements, estimates of sun exposure, skin type, clothing and SNPs. Owing to limited resources, the sample size of this study is small. This had limited the number of SNPs that could be studied in the present study. Nonetheless, we have selected two SNPs (rs4588 and rs7041) that most reported associated with 25OHD in the previous studies. The sample size of the current study had a sufficient power to detect the prevalence and the associations of the demographics, lifestyle and VDBP SNPs (or diplotypes) with maternal VDD.

The high prevalence of maternal VDD reported in this study indicates the need for urgent development and implementation of strategies to improve maternal vitamin D status. In Malaysia, where sunlight is available throughout the year, advocating increasing sunlight exposure will be a cost-effective measure. Nonetheless, as a large majority of Malaysian women are veiled, advocating sun exposure may not increase the 25OHD level in the majority of them. Veiled is a non-modifiable risk factor almost all of the Malay women are veiled for the religious requirement. Given that the food source of vitamin D is limited, it appears that the potential strategy to increase 25OHD level in pregnant women is vitamin D supplementation. To support this, our study found a significant association of vitamin status with vitamin D intake from the supplement. However, less than half (40.1%) of the study participants took a vitamin D containing supplement, and among the supplement users, prenatal multivitamin was the most prevalent supplement (66.7%) consumed. Most of these pregnant women obtained their prenatal multivitamins (containing 10 μg of vitamin D per capsule) from public health clinics during their antenatal visit while few of them bought the supplement themselves. It should be noted that in the Malaysian public health clinic, the provision of prenatal multivitamin D is not universal. However, the provision is dependent on the availability and the requirement of pregnant women. Taken together, this

study suggests that the universal provision of a prenatal multivitamin may be effective in improving the vitamin D status of pregnant women. Investigating the effectiveness of supplement pregnant women in randomised controlled trials could be the next step if to develop national recommendations or policies for supplementation. Additionally, the significant association of *GC* SNPs (and *GC* diplotypes) with maternal VDD showed in the present and the previous study suggests that supplementation should be more personalised based on genotype and risk factors assessment, particularly in pregnant women.

Conclusions

The finding indicates that half of the studied pregnant women were VDD (25OHD < 30 nmol/L). Despite the abundance of sunlight in Malaysia, sun exposure did not contribute to maternal vitamin D status. The analysis showed that the risk of VDD was more than 4 times higher in veiled than unveiled women. Likewise, vitamin D intake from food and supplements were significantly associated with maternal VDD. The contribution of *GC* rs4588 and rs7041 (or *GC* diplotypes) to maternal VDD was minimal but was significant. Taken together, this study suggests the need for urgent development and implementation of strategies, and supplementation appears to be the best strategies to improve the vitamin D status of the study population.

Declarations

Ethics approval and consent to participate:

Ethical approval was obtained from the Medical Research and Ethics Committee Ministry of Health Malaysia (MREC) with the ID: NMRR-15-786-24865. The written informed consent was obtained from pregnant women prior to data collection if they agreed to participate in the study.

Consent for publication:

Not applicable

Availability of data and materials:

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests:

The authors declare that they have no competing interests.

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Author Contributions:

SPL, RS and KHL contributed to conceptualization and methodology of the study. SPL and MT contributed to the funding acquisition. SSL, MT and KFR involved in data Curation. SSL administered the study, performed the statistical analyses and wrote the original manuscript. All the authors critically revised the manuscript and approved the final version.

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Figures

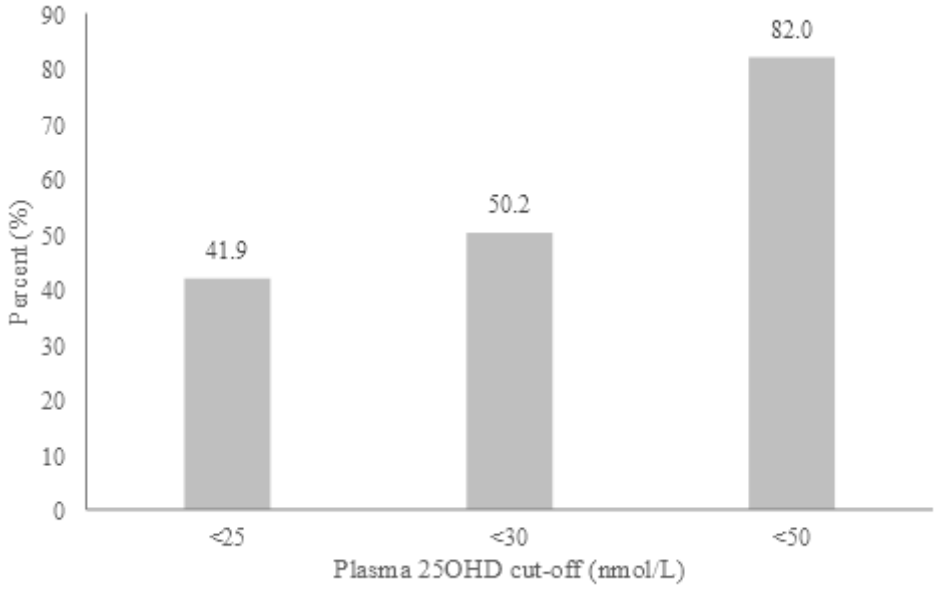

Figure 1

Maternal vitamin D status