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Genetic variants and non-genetic factors associated with a high prevalence of vitamin D deficiency in full-term neonates in Malaysia

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ABSTRACT

A high prevalence of vitamin D deficiency has been reported in Malaysian pregnant women, indicating that neonates at birth are at increased risk of vitamin D deficiency. Factors including low dietary vitamin D intake and single nucleotide polymorphisms in the vitamin D metabolism genes have been associated with this deficiency in the population. However, there was limited data available regarding the prevalence of neonatal vitamin D deficiency and the factors associated with it among neonates in Malaysia. Therefore, this study aimed to determine the prevalence of vitamin D deficiency and explore the associations between maternal and neonatal gene polymorphisms, as well as non-genetic factors and neonatal vitamin D deficiency. A total of 217 mother-neonate dyads were recruited for this study. Data on skin type, maternal sun exposure, dietary intake, as well as maternal and neonatal 25-hydroxyvitamin D (25OHD) concentrations were collected. Maternal and neonatal vitamin D Receptor (VDR) SNP (rs2228570) and Group-specific component (GC) SNPs (rs4588 and rs7041) genotypes were determined using high-resolution melting (HRM) and restriction fragment length polymorphism, respectively. The results showed that 60.4%, 71.4% and 95.4% of neonates had cord blood 25OHD levels below 25 nmol/L, 30 nmol/L and 50 nmol/L, respectively. After adjusting for the maternal vitamin D status, the maternal *VDR* rs2228570 GG genotype was significantly associated with neonatal vitamin D deficiency (25OHD<30 nmol/L) (aOR = 2.63, 95% CI: 1.18–5.87, p = 0.018). Maternal vitamin D supplement intake was found to be a protective factor. However, maternal and neonatal vitamin D binding protein (VDBP) SNPs were not associated with neonatal vitamin D deficiency. The high prevalence of neonatal vitamin D deficiency reported in this study indicates the urgent need for the development and implementation of strategies to improve neonatal vitamin D status. The findings suggest that maternal supplementation may be an effective approach to enhance the vitamin D status of neonates.

1. Introduction

Vitamin D deficiency during pregnancy is a public health concern worldwide with the global prevalence of maternal vitamin D deficiency ranging from 51% to 100% [1]. The widespread vitamin D deficiency among pregnant women can be attributed to factors including limited sun exposure, skin pigmentation, clothing choices, season variations, inadequate dietary vitamin D intake and genetic variants [2–6]. Poor maternal vitamin D status can lead to neonatal vitamin D deficiency at birth, as *in utero,* the fetus depends entirely on the transplacental transfer of 25-hydroxyvitamin D (25OHD) for its vitamin D supply and stores [7]. Neonatal vitamin D deficiency at birth has been linked to an

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increased risk of preterm birth, neonatal hypocalcemia, neonatal respiratory distress syndrome, transient early wheezing and hospitalization during the first year of life due to acute respiratory infection or acute gastroenterocolitis [8,9]. Maternal and neonatal vitamin D deficiency can be prevented with antenatal or neonatal vitamin D supplementation.

In high-latitude countries such as the United Kingdom [10], United States [11], Canada [12], Germany [13] and the Nordics regions [14], where vitamin D deficiency prevalence is high, there is a formal recommendation for routine vitamin D supplementation for high-risk pregnant women and neonates, particularly during winter months. While epidemiological studies consistently showed a high prevalence of vitamin D deficiency among pregnant women in sunny countries, including Malaysia [3,15–17], Indonesia [18] and Thailand [19], there is currently no formal recommendation for antenatal or neonatal vitamin D supplementation in these countries. Until recently, there was a paucity of data to support and inform the recommendation of antenatal or neonatal vitamin D supplementation and other preventive measures in these regions.

Despite the crucial role of neonatal vitamin D status in determining the health outcomes of neonates, there has been relatively little focus on investigating the prevalence and factors associated with vitamin D deficiency at birth. Most studies describing the prevalence and determinants of neonatal vitamin D deficiency were from European countries. Many previous cord blood studies have been limited by small sample sizes ($n < 100$), with statistical analyses restricted to bivariate analysis only. While previous studies have explored a wide range of factors associated with neonatal vitamin D deficiency [20–26], few studies have investigated the associations between maternal and neonatal single nucleotide polymorphisms (SNPs) in the vitamin D metabolism pathway and non-genetics factors on neonatal vitamin D deficiency [20,27]. Understanding the prevalence and factors associated with neonatal vitamin D deficiency at birth may provide evidence to inform the development of preventive strategies, such as vitamin D supplementation recommendations for pregnant women and neonates. Therefore, the present study aimed to (1) assess the prevalence of neonatal vitamin D deficiency at birth (as measured in umbilical cord blood) and (2) determine the associations between maternal and neonatal SNPs in vitamin D metabolism and non-genetic factors with vitamin D deficiency at birth. In particular, three well-studied missense single-nucleotide polymorphisms (SNPs) were selected: GCrs7041, GCrs4588 and VDRrs2228570. These SNPs have been previously associated with 25OHD concentration and vitamin function in genetic association studies.

2. Material and methods

2.1. Study design and participants

A detailed description of the research methodology has been described previously [3]. In brief, healthy mother-neonate dyads were recruited between October 2015 through February 2017 at the labor suite of Hospital Serdang, Selangor, Malaysia. The inclusion criteria were Malaysian pregnant women, aged between 19 and 40 years, in full-term pregnancy (gestational week $\geq$37 weeks), with singleton live births. Pregnant women meeting the following criteria were excluded from participation: pre-existing hyperthyroidism, rheumatoid arthritis, diabetes mellitus, gestational diabetes, preeclampsia, pregnancy-induced hypertension and a history of bone and renal disorders. Neonates with congenital anomalies were also excluded from this study. Informed consent was obtained prior to enrollment. This study was approved by the Medical Research and Ethics Committee Ministry of Health Malaysia (MREC) (NMRR ID: 15-786-24865) and conducted in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Data on the prevalence of vitamin D deficiency in cord blood in Malaysia was not available. Therefore, a prevalence of 50% was assumed, as it would give the largest sample size. Using an estimated prevalence of 50 % ($p = 0.5$) and a precision of 7% ($d = 0.07$) with a 95% confidence interval ($Z = 1.96$), the sample size was calculated to be 196 using the formula: $n = Z^2P(1 - P)/d^2$. An addition of 20% was factored in for non-responses, resulting in a minimum sample size requirement of 245 pairs of mother-neonate.

2.2. Measurement of plasma 25-hydroxyvitamin D

Five milliliters of venous blood were drawn from pregnant women on the day of labor, while umbilical cord blood was collected from the severed umbilical cord end after clamping. Plasma and buffy coat samples were stored at −80 °C until further analysis. Plasma 25OHD concentrations were determined using in-house validated ultra-high-performance liquid chromatography (UHPLC) [28] with an inter-assay coefficient of variation of 6–7%. As there is no widely accepted definition of vitamin D deficiency, multiple cut-offs (<25, <30 and < 50 nmol/L) were used in the present study to describe vitamin D deficiency in comparison with previous studies. For the analysis of factors associated with neonatal vitamin D deficiency, a cut-off value of 30 nmol/L, as proposed by the National Academy of Medicine (formerly known as the Institute of Medicine [29]), which is associated with an increased risk of vitamin D deficiency, was used.

2.3. Data collection

Data on maternal factors were collected through an interviewer-administered questionnaire. Data collected included maternal socio-demographics information, sun exposure, skin type and vitamin D intake. Maternal characteristics, including age at delivery, parity, pre-pregnancy Body Mass Index (BMI) and gestational weight gain; and neonatal characteristics, including birth date, gestational age at birth and sex, were extracted from hospital electronic medical records and antenatal records.

Maternal skin type was estimated using the Fitzpatrick scale, which classifies skin type into six different skin phototypes based on the women's skin color and skin tanning evaluation. Due to the small sample size for each skin type subset, skin color types were dichotomized into light skin color (Fitzpatrick scale I to III) and dark skin color (Fitzpatrick scale IV and V) for the present study [30]. Sun exposure was assessed using a questionnaire adapted from Hall, Kimlin [31], while maternal daily vitamin D intake from both food and supplements was estimated using a validated vitamin D-specific semi-quantitative Food Frequency Questionnaire (FFQ) [32].

2.4. Genotyping

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

SNPs were chosen based on prior literature. Three SNPs *VDR* rs2228570, *GC* rs7041 and *GC* rs4588 were selected because they are nonsynonymous or functional SNP, whereby a single nucleotide variation leads to amino acid alteration. Moreover, these SNPs are common in the study population with a minor allele frequency (MAF) of $\geq$20% in East Asian (EAS) and South Asian (SAS) populations as reported in the 1000 Genome Project [33].

Maternal and neonatal *GC*rs4588 and rs7041 genotyping were performed using the restriction fragment length polymorphism (RFLP) technique. In brief, PCR amplification was performed using a thermocycler (Thermo Fisher Scientific, USA), and the resulting PCR product, a 483-bp fragment, was then separately digested using the restriction enzyme *Hae*III (for rs7041) and *Sty*I (for rs 4588) (New England Biolabs Inc., USA). The digested products were subsequently analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide and

compared with the 100-bp DNA size marker (Vivantis Technologies Sdn Bhd, Malaysia). For rs7041, A > C transversion creates a restriction site for *Hae*III, yielding 295 bp and 167 bp bands. Similarly, a G > T transversion in rs4588 creates a restriction site for *Sty*I, resulting in 302 bp and 156 bp bands (Fig. 1).

*VDR*rs2228570 genotyping was performed using High-Resolution Melting (HRM) analysis on the LightCycler 480 Instrument (Roche, Switzerland). The LightCycler® 480 Gene Scanning software was used to identify sequence variants. Approximately 15% of samples were randomly selected for DNA sequencing analysis to validate the genotyping results. The concordance rate of sequencing results and HRM results was 96.9%. For rs4588 and rs7041, all the sequencing outcomes corresponded with the genotypes predicted by RFLP analysis, achieving a concordance rate of 100%.

2.5. Statistical analyses

Statistical analysis was performed using SPSS version 26.0 (SPSS Inc., Chicago, USA). Hardy-Weinberg Equilibrium (HWE) for each SNP was examined using the Chi-square test, indicating that the genotype distribution for all the SNPs was within Hardy-Weinberg equilibrium ($\chi^2 < 0.3841$, $p > 0.05$)

Univariate binary logistic regression was initially performed to assess the individual associations between maternal and neonatal factors and neonatal vitamin D deficiency (25OHD <30 nmol/L). The associations between maternal and neonatal *VDR* rs2228570, *GC* rs4588 and rs7041 with neonatal vitamin D deficiency (25OHD <30 nmol/L) were analyzed using multivariate logistic regression. Variables with p-value <0.1 in univariate analyses were included in multivariate analyses [34]. Statistical significance was set at a p-value <0.05 for all analyses.

3. Results

This study included 217 mother-neonate dyads with complete SNP data and cord blood 25OHD measurement. The mean gestational age at birth for the neonates was 39 ± 1 week with an equal proportion of male (49.3%) and female neonates (50.7%) (Table 1). Not more than half (40.1%) of the pregnant women took a multivitamin containing vitamin D during pregnancy. As shown in Table 2, the median cord blood plasma 25OHD concentration was 22.0 nmol/L (Q_1-Q_3 = 15.5–31.0 nmol/L). Of these, 207 (95.4%), 155 (71.4%) and 131 (60.4%) of neonates had plasma 25OHD levels <50, <30 and < 25 nmol/L, respectively.

Univariate logistic regression analyses showed that the maternal factors associated with an increased risk of neonatal vitamin D deficiency included wearing veiled clothing and maternal vitamin D deficiency (Table 3). Conversely, nulliparity, gestational weight gain and vitamin D intake from supplements were protective factors. Similar observations were noted when defining vitamin D deficiency using a cutoff of 25 nmol/L (Supplementary Table S1 and Table S2). None of the maternal SNPs was associated with neonatal vitamin D deficiency in the univariate analyses. More neonates born between April and November had 25OHD <30 nmol/l than those born between December and March of the year (Table 4). No associations were found between neonatal *VDR* and *GC* SNPs with cord vitamin D deficiency (25OHD<30 nmol/L).

In the multivariate analysis (Table 5), maternal *VDR* rs2228570 was significantly associated with cord blood vitamin D deficiency (25OHD <30 nmol/L), even after adjusting for factors including vitamin D intake from the supplement, nulliparity, veiled clothing and maternal vitamin D status. Neonates born to mothers with vitamin D deficiency had about seven times the risk of being deficient in vitamin D compared to those born to mothers with normal vitamin D status. Additionally, the risk of deficiency was approximately two times higher in neonates born to mothers with the *VDR* rs2228570 GG genotype. These associations remained significant even after controlling for neonatal *VDR* rs2228570 genotypes.

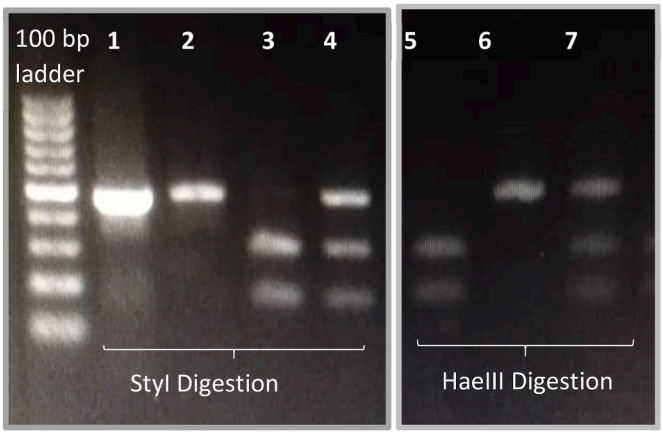

Fig. 1. Visualisation of *GC* rs4588 and rs7041 genotypes
Lane 1, undigested PCR product (483 bp); Lane 2, rs4588 homozygous wild type (GG); Lane 3, rs4588 homozygous mutant type (TT); Lane 4, rs4588 heterozygous (GT); Lane 5, rs7041 homozygous mutant type (CC); Lane 6, rs7041 homozygous wild type (AA); Lane 7, rs7041 heterozygous (AC).

Table 1
Maternal and neonatal characteristics.

Characteristics	n (%) or Mean ± SD
Maternal	
Age (years)	29 ± 4
Ethnicity	
Malay	187 (86.2)
Chinese	20 (9.2)
Indians and Others	10 (4.6)
Education	
Secondary and lower	94 (43.3)
Tertiary and higher	123 (56.7)
Household Income (per month)	
≤ RM3000	64 (30.0)
RM3001-RM5000	94 (44.1)
≥ RM5001	55 (25.9)
Pre-pregnancy body mass Index (kg/m^2)	23.7 ± 5.1
Gestational weight gain (kg)	10.6 ± 5.0
Parity: Nulliparous	58 (26.7)
Use of vitamin D containing supplement	87 (40.1)
Neonatal	
Infants sex: Male	107 (49.3)
Gestational age at birth (weeks)	39.1 ± 1.1
Month of birth	
December–March	76 (35.0)
April–November	141 (65.0)
Birth weight (kg)	3.1 ± 0.4
Crown-heel length at birth (cm)	48.3 ± 1.8
Head circumference (cm)	32.8 ± 1.2

Table 2
Umbilical cord blood plasma 25OHD concentrations (n = 217).

Vitamin D level and status	n	%	Median (Q1, Q3)
Plasma 25OHD nmol/L			22.0 (15.5, 31.3)
Plasma 25OHD (categorized)			
<30 nmol/L	155	71.4	
≥30 nmol/L	62	28.6	
Plasma 25OHD (categorized)			
<25 nmol/L	131	60.4	
25–49.99 nmol/L	76	35.0	
≥50 nmol/L	10	4.6	

25OHD, 25-hydorxyvitamin D.

Table 3
Univariate analysis of associations of neonatal vitamin D deficiency (25OHD<30 nmol/L) with maternal factors, VDBP and VDR gene polymorphism.

Variables	Umbilical cord blood 25OHD		Crude OR	p-value
	<30 nmol/L (n = 155)	≥30 nmol/L (n = 62)		
Maternal age	29.2 ± 4.1	28.1 ± 4.3	1.07 (0.99, 1.15)	0.069
Ethnicity				
Chinese	11 (55.0)	9 (45.0)	0.45 (0.18, 1.15)	0.094
Malay and Indians	144 (73.1)	53 (26.9)	Reference	
Maternal education				
Secondary and below	71 (75.5)	23 (24.5)	1.4 (0.78, 2.62)	0.243
Tertiary and above	84 (68.3)	39 (31.7)		
Household income				
≤ RM3000	48 (75.0)	16 (25.0)	1.46 (0.66, 3.24)	0.353
RM3001-RM5000	67 (71.3)	27 (28.7)	1.21 (0.59, 2.48)	0.608
≥ RM5001	37 (67.3)	18 (32.7)		
Parity				
Nulliparous	35 (60.3)	23 (39.7)	0.50 (0.26, 0.94)	0.031
Multiparous	120 (75.5)	39 (24.5)	Reference	
Pre-pregnancy BMI	24.1 ± 5.1	22.9 ± 5.0	1.05 (0.99, 1.12)	0.121
GWG	10.2 ± 5.1	11.9 ± 4.6	0.93 (0.88, 0.99)	0.024
Vitamin D intakes (μg/day)				
From food	8.1 ± 4.9	8.7 ± 5.4	0.98 (0.92, 1.04)	0.444
From Supplements	3.0 ± 4.8	6.0 ± 6.6	0.91 (0.87, 0.96)	0.001
Skin type				
1, II & III	73 (75.3)	24 (24.7)	0.71 (0.39, 1.29)	0.263
IV, V &VI	82 (68.3)	38 (31.7)	Reference	
Veiled (Yes)				
Yes	133 (74.3)	46 (25.7)	2.10 (1.02, 4.35)	0.045
No	22 (57.9)	16 (42.1)	Reference	
Maternal 25OHD				
<30 nmol/l	98 (89.9)	11 (10.1)	7.97 (3.8, 16.5)	0.001
≥30 nmol/l	57 (52.8)	51 (47.2)	Reference	
VDR rs2228570				
AA + AG	96 (67.6)	46 (32.4)	Reference	0.088
GG	59 (78.7)	16 (21.3)	1.77 (0.92, 3.40)	
GC rs4588				
GG	99 (73.3)	36 (26.7)	Reference	0.426
GT + TT	56 (68.3)	26 (31.7)	0.78 (0.43, 1.43)	
GC rs7041				
AA	65 (73.0)	24 (27.0)	Reference	0.663
AC + CC	90 (70.3)	38 (29.7)	0.87 (0.48, 1.60)	

25OHD, 25-hydorxyvitamin D; GWG, gestational weight gain; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

4. Discussion

Vitamin D deficiency at birth has been associated with numerous adverse health outcomes. Hence, this study aims to determine the prevalence and factors, both genetic and non-genetic factors associated with neonatal vitamin D deficiency at birth, as measured in umbilical cord blood. Our findings revealed a high prevalence of vitamin D deficiency among Malaysian neonates at birth. As expected, maternal vitamin D status was the most significant predictor of umbilical cord blood vitamin D status. Additionally, neonatal vitamin D deficiency was also associated with maternal vitamin D supplementation. Interestingly, our analysis showed that maternal *VDR* rs2228570 is also associated with cord blood vitamin D deficiency. However, no significant associations were observed between maternal and neonatal *VDBP* SNPs and neonatal vitamin D deficiency.

Table 4
Univariate analysis of associations between neonatal vitamin D deficiency (25OHD<30 nmol/L) with neonatal factors, VDBP and VDR gene polymorphism.

Variables	Cord blood 25OHD		Crude OR (95% CI)	p-value
	<30 nmol/L	≥30 nmol/L		
Infant sex				
Male	79 (73.8)	28 (26.2)	0.79 (0.33, 1.43)	0.440
Female	76 (69.1)	34 (30.9)	Reference	
Gestational age at birth (weeks)	39.1 ± 1.1	39.2 ± 1.1	0.91 (0.70, 1.18)	0.477
Month of birth				
Dec–Mar	47 (61.8)	29 (38.2)	0.50 (0.27, 0.91)	0.023
Apr–Nov	108 (76.6)	33 (23.4)	Reference	
VDR rs2228570				
AA + AG	101 (70.6)	42 (29.4)	Reference	0.717
GG	54 (73.0)	20 (27.0)	1.12(0.60, 2.10)	
GC rs4588				
GG	61 (73.5)	22 (26.5)	Reference	0.596
GT + TT	94 (70.1)	40 (29.9)	1.18 (0.64, 2.18)	
GC rs7041				
AA	85 (72.6)	32 (27.4)	Reference	0.667
AC + CC	70 (70.0)	30 (30.0)	0.89 (0.49, 1.59)	

25OHD, 25-hydorxyvitamin D; VDR, vitamin D receptor.

Table 5
Multivariate analysis of factors associated with neonatal vitamin D deficiency.

Variables	aOR	95% CI	p-value
Maternal age	1.04	0.95, 1.15	0.398
Vitamin D intake from supplements	**0.93**	**0.87, 0.99**	**0.026**
Nulliparous	0.49	0.21, 1.16	0.104
Veiled clothing (Yes)	1.81	0.74, 4.41	0.190
Maternal *VDR* rs2228570 (GG)	2.63	**1.18, 5.87**	**0.018**
Maternal 25OHD <30 nmol/L	7.36	**3.31, 16.37**	**0.0001**
GWG	0.95	0.89, 1.03	0.210
Month of birth	0.59	2.83, 1.21	0.147

25OHD, 25-hydorxyvitamin D; GWG, gestational weight gain; VDBP, vitamin D binding protein; VDR, vitamin D receptor.

The prevalence of neonatal vitamin D deficiency reported in this study (95.4% with 25OHD <50 nmol/L) was comparable with a recent local finding by Mustapa Kamal Basha, Abdul Majid [35], where a prevalence of 96.1% was reported. Similarly, the prevalence was comparable to those reported in neighboring countries such as Thailand [26] and Indonesia [27], albeit higher relative to large-scale studies in China [36], Europe [4,7,37] and America [22,38].

Consistent with previous studies [7,21,26,39–41], our study found that maternal vitamin D status primarily determines neonatal vitamin D deficiency. This is expected as the fetus relies entirely on the maternal vitamin D for the transplacental transfer of 25OHD to meet their vitamin D needs. As human breast milk typically contains minimal vitamin D and is usually insufficient to prevent rickets in the breastfed infant [42,43], neonates rely on their vitamin D stores and dietary intake for their vitamin D supply [44]. Studies have shown that infants who were exclusively breastfeeding for six months of age were at increased risk of vitamin D deficiency by that age. Therefore, our findings, which demonstrate the independent association of maternal supplementation

with neonatal vitamin D deficiency, support the recommendation for pregnant women to receive supplementation and ensure adequate intake from their diet to reduce the risk of vitamin D deficiency at birth. In support of this, randomized control trials consistently reported that vitamin D supplements effectively increase umbilical cord venous blood and neonatal 25OHD compared to placebos [45–47].

Previous studies have examined the associations between maternal vitamin D-related SNPs and maternal vitamin D status. However, there is limited evidence regarding the association between these SNPs and cord blood vitamin D status. In a study by Moon, Harvey [48], the association of maternal and neonatal SNPs related to vitamin D metabolism pathway (*DHCR7* rs12785878, *CYP2R1*rs10741657, *CYP24A1* rs6013897, *GC* rs2282679) with umbilical cord blood 25OHD levels were analyzed. Their analysis revealed that the association of cord 25OHD concentration with maternal rs12785878 (*DHCR7)* remained significant, while maternal rs2282679 (*GC)* attenuated after adjustment for neonatal SNP. Similarly, a multi-ethnic Australian study observed an association between fetal *GC* SNP rs22282679 (a proxy of rs4588) and neonatal 25OHD levels [24]. However, the present study did not find evidence of associations between VDBP SNP with cord vitamin D deficiency. A potential explanation for this discrepancy could be attributed to variations in the outcomes analyzed. The studies by Moon, Harvey [48] and Smith, Sun [24] analyzed the associations between SNPs and cord 25OHD concentrations, a continuous variable. However, the present study focused on cord blood vitamin D deficiency, defined as 25OHD <30 nmol/L. Supporting this, our findings align with a recent study by Karras, Dursun [49] in Southern Europe, which employed different maternal and neonatal 25OHD cut-offs to define deficiency. The study concluded that maternal and neonatal VDBP polymorphism is not associated with neonatal vitamin D status at birth.

VDR SNPs have been extensively studied for their associations with various adverse health outcomes, including pregnancy and birth outcomes such as preterm birth, low birth weight, small-for-gestational -age (SGA), as well as a wide range of pregnancy complications [50–53]. However, an early study by Engelman, Fingerlin [54] found a significant association between *VDR* rs10783219 with the 25OHD level in San Antonio Hispanics. This finding is consistent with our results demonstrating that maternal *VDR* rs2228570 is independently associated with cord 25OHD deficiency. *VDR* rs2228570 is a missense SNP (non-synonymous SNP) where an A to G allele transversion creates an alternative translation start site, resulting in a VDR protein that is three amino acids shorter than the wild type. The shorter VDR isoform may be associated with increased transcriptional activity [55]. It seems plausible that this increased activity could influence the transplacental transfer of 25OHD, thereby increasing the risk of cord blood vitamin D deficiency.

A major strength of this study is its comprehensive examination of a wide range of maternal and neonatal factors associated with neonatal vitamin D deficiency. Additionally, the study took a further step by determining the association of maternal vitamin D-related SNPs with neonatal vitamin D status, aiming to elucidate the mechanisms underlying the maternal-fetal transfer of vitamin D. The data on the prevalence of vitamin D deficiency in neonates contribute to the existing knowledge on neonatal vitamin D deficiency in Southeast Asia. One limitation of the study was the incapability of the current 25OHD measurement method to quantify C-3 epimers. However, this limitation does not diminish the validity of the assay. It is noteworthy that while LC-MS/MS might not be available and affordable for laboratories in developing countries, a vitamin D assay capable of providing accurate, reproducible results is sufficient.

5. Conclusions

The high prevalence of neonatal vitamin D deficiency highlighted in this study indicates the urgent need for the development and implementation of strategies aimed at improving neonatal vitamin D status. Our findings strongly suggest that maternal supplementation could potentially improve neonatal vitamin D levels within the Malaysian context. Furthermore, extending research on maternal and neonatal vitamin D status to a nationwide or multi-center study with a larger and more representative sample than that of the present study is warranted. Such a comprehensive study on a national scale could provide valuable insights into the need to develop formal recommendations or policies regarding the supplementation of pregnant women and infants. Our findings also demonstrated the potential influence of maternal *VDR* SNP on the transplacental transfer of 25OHD. Further investigation and analysis are imperative to elucidate the mechanism underlying the placental transfer of 25OHD.

CRediT authorship contribution statement

Siew-Siew Lee: Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. **King-Hwa Ling:** Formal analysis, Supervision, Conceptualization, Writing – review & editing. **Raman Subramaniam:** Conceptualization, Supervision, Methodology. **Maiza Tusimin:** Supervision, Data curation, Funding acquisition. **Kartini Farah Rahim:** Supervision, Data curation, Writing – review & editing. **Su-Peng Loh:** Conceptualization, Funding acquisition, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hnm.2024.200259.

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