ORIGINAL ARTICLE

Association of Maternal and Cord Plasma Total, Free and Bioavailable 25-Hyrodroxyvitamin D with Neonatal Anthropometric Measurements at Birth: A Preliminary Study in a Private Hospital

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ABSTRACT

Introduction: 25-hydroxyvitamin D (25OHD) is the principal biomarker of vitamin D status. In circulation, 25OHD is primarily bound to vitamin D binding protein (VDBP), leaving a small proportion bound to albumin and as free form. Previous studies have suggested that free 25OHD is better correlated with health outcomes. However, in pregnancy where VDBP level is extremely elevated, the correlations between free 25OHD with health outcomes are far from conclusive. Here we show the associations of maternal and cord total, free and bioavailable 25OHD concurrently with neonatal anthropometric measurements in healthy pregnant mothers-neonates pairs. **Method:** Total 25OHD level was measured by using chemiluminescent immunoassay. Free and bioavailable 25OHD were calculated using published mathematical models. **Results:** The results showed that birth weight and head circumference were negatively associated with maternal total 25OHD but not significantly associated with free and bioavailable 25OHD. There were no significant associations between cord total, free and bioavailable 25OHD with any of the neonatal anthropometric measurements. **Conclusion:** The outcomes of this study should encourage further research in a larger sample size. Notably, future research could lead to the establishment of causative relationships and plausible mechanisms between maternal and cord 25OHD with neonatal anthropometric measurements.

Keywords: Vitamin D; Vitamin D binding protein; Bioavailable 25OHD; Free 25OHD; Anthropometric measurements

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INTRODUCTION

25-hydroxyvitamin D (25OHD) is the prohormone of vitamin D. It is synthesised in the liver and then hydrolysed in the kidney to the active form of vitamin D-1,25-dihydroxyvitamin D (1,25OHD). Being the major circulating vitamin D metabolite and having a relatively long half-life, serum or plasma 25OHD has been used as the principal marker for vitamin D status (1, 2). In the circulation, about 80-90% of 25OHD is bound to vitamin D binding protein (VDBP) and about 12% is bound to albumin, leaving less than one percent present as free form (3). The affinity of 25OHD for albumin is substantially weaker than that observed for VDBP. Hence, the albumin bound 25OHD plus free 25OHD is referred to as bioavailable 25OHD (4).

As the affinity of VDBP to 25OHD is high, VDBP bound 25OHD is not available for passive diffusion into cells. The uptake of VDBP bound 25OHD is dependent on a transmembrane protein, megalin. In contrast, the relatively low affinity of albumin to 25OHD makes free and bioavailable 25OHD more diffusible. Thus, free and bioavailable 25OHD can passively diffuse into cells independent on megalin (4). Several studies

have revealed that free and bioavailable 25OHD were better correlated to the bone health markers and non-calciotropic health outcomes than total 25OHD. On the contrary, there are studies showing that the associations between outcomes with free 25OHD and bioavailable 25OHD were not stronger than the associations with total 25OHD (5).

The concentration of VDBP is two to three folds higher in pregnancy than in the non-pregnant state (6-8). The increase could be attributed to the stimulation of VDBP production by estrogen (9), but the functional significance of the elevation in VDBP so far remains unclear. Previous studies have consistently reported that the increase in maternal VDBP is concurrent with an increase in maternal total 1,25OHD. As a result, free 1,25OHD level in the circulation is maintained (6, 7). However, the increase in 1,25OHD is expected to increase 25OHD expenditure, thus decreasing maternal total 25OHD. The increase of VDBP may lead to a decrease in free and bioavailable 25OHD (10, 11). This can conserve bound 25OHD (8) for placental metabolism and transfer to fetus. In a review by Bikle et al.(5), it has been demonstrated that total 25OHD level is likely to be influenced by VDBP. They suggested that measuring free 25OHD may better reflect vitamin D sufficiency in pregnancy. Neonatal size at birth has been shown to affect perinatal morbidity and is associated with the risk of metabolic syndrome and disease development later in life (12, 13). In recent years, a growing body of literature has investigated the associations of vitamin D with neonatal size at birth. Some studies have found significant associations between maternal 25OHD with neonatal anthropometric measurements (14-16), while others did not (17, 18). Likewise, review and meta-analyses on the associations between maternal and cords 25OHD with neonatal size at birth have highlighted the inconsistency of the associations results (19, 20). This inconsistency can be explained by the heterogeneity of the study design between these studies: gestational age at sampling, study population, geographical region and controlled variables. Nevertheless, it is important to mention that most of these studies investigated the associations of 25OHD in maternal blood or cord blood with neonatal outcomes. While maternal 25OHD and cord 25OHD can confound each other for the associations with neonatal outcomes, limited studies assessed the associations concurrently in both maternal and cord blood.

The inconsistency of vitamin D associations with neonatal anthropometric measurements could be due to free or bioavailable 25OHD rather than the total 25OHD is associated with neonatal anthropometric measurements. Exploring the correlation between free and bioavailable 25OHD may partly unravel and explain the previous inconsistency in the associations of vitamin D with neonatal anthropometric measurements at birth. However, to date, there has been limited evaluation on the concentration of free and bioavailable 25OHD with neonatal anthropometric measures at birth. Hence, this study is aimed to determine the relationship between maternal and cord total, free and bioavailable 25OHD. Furthermore, we examined the associations between maternal and cord total, free and bioavailable 25OHD with fetal last ultrasound biometry and neonatal anthropometric measurements.

MATERIALS AND METHODS

Participants

Pregnant women delivering at Pantai Hospital, Kuala Lumpur, Malaysia during the study period were invited to participate in this study. Subjects were included if the following criteria were met: i) Malaysian, ii) aged 19 to 40 years, iii) singleton pregnancy, iv) gestational age ≥ 37 weeks. Pregnant women who were diagnosed with pre-existing systemic disease or pregnancy complications or had a history of bone and renal disorders, as well as infants born with congenital anomalies, were excluded from this study. The study was approved by the Ethics Committee for Research Involving Human Subjects (JKEUPM) and was conducted in accordance with the Helsinki Declaration. Written consent was obtained from all participants before they took part in the study.

Power analysis was performed using G*Power software. Prior to this study, information on the coefficient of determinants between 25OHD and neonatal outcomes was unavailable. In general, the coefficient of determinants of 0.3 is considered sizeable (21). Thus, using a conservative estimate of coefficient of determinants of 0.3 with a 95% confidence interval, we calculated that present sample size of 77 had a power of 77% to detect significance in the associations.

Measurement

Information on maternal age, pre-pregnancy weight, height, household income, education level, ethnicity and obstetric history were obtained through self-administered questionnaire. Information on first booking (date, weeks and ultrasound scan) and last booking (dates, weeks and biparietal diameter) were obtained from the medical record book of each study participant. Gestational age was determined by last menstrual period (LMP) and confirmed by the first dating scan. The anthropometric measurements of newborn which include birth weight, head circumference, length and sex were obtained from hospital birth records. Ponderal index, an indicator of infant fatness was calculated using equation 1 (22).

$$\text{Ponderal index} = \frac{\text{birth weight (kg)}}{\text{crown-to-heel length (m}^3\text{)}} \quad (1)$$

Blood Collection and Laboratory Analysis

Five milliliters of venous blood from the pregnant mother was collected prior to delivery and 5 mL of cord blood from the severed umbilical cord was collected

after delivery of the newborn, but before expulsion of placenta. These blood samples were centrifuged at 3700 rpm for 15 minutes at 4 °C, and the plasma aliquots were kept at -80 °C until analysis. Plasma total 25OHD was measured using chemiluminescent immunoassay (CLIA) via LIAISON analyser (DiaSorin Inc, Stillwater, MN, USA), an automated platform. The assay involved dissociation of 25OHD from its binding protein and binding of 25OHD to specific antibody on the solid phase, followed by the addition of tracer (vitamin D linked to an isoluminol derivative). Lastly, the starter reagents were added to initiate a flash chemiluminescent reaction. The light signal was measured by a photomultiplier as relative light units. The mean coefficient of variance (CV) for the assay was 5-6% and the limit of quantification (LoQ) was 12.5nmol/L. Maternal and neonatal plasma 25OHD levels were categorised according to previously described categories of vitamin D status (23-25): as vitamin D deficiency (<25 nmol/L), vitamin D insufficiency (25-50 nmol/L) and vitamin D sufficiency (>50 nmol/L). The circulating Vitamin D binding protein (VDBP) was measured by using the Quantikine Human Vitamin D Binding Protein Immunoassay Kit (R&D Systems, Minneapolis, USA) according to the manufacturer's instructions. The mean CV for the VDBP assay was 6%. Albumin was measured using immunoturbidimetric assay (Roche cobas c311, Mannheim, Germany). The concentration of free 25OHD was calculated by using the equation from Powe et al. (26) (equation 2). The affinity binding constants for 25OHD with albumin and VDBP used were 6 X 105 M-1 and 7 X 108 M-1, respectively. Bioavailable 25OHD was calculated as sum of free 25OHD and albumin-bound 25OHD using equation 3 (27).

$$\text{Free 25OHD} = \frac{\text{Total 25OHD}}{1 + (6 \times 10^5 \times \text{Albumin}) + (7 \times 10^8 \times \text{VDBP})} \quad (2)$$

$$\text{Bioavailable 25(OH)D} = \text{Free 25(OH)D} + \text{Albumin bound 25(OH)D} \quad (3)$$

Statistical Analysis

All the statistical analyses were performed using IBM SPSS version 21.0. The characteristics of the study participants were described by number and percentage for categorical variables. Continuous variables were tested for normality using Smirnov-Kolmogorov test and skewness (≤+2 or ≤-2) and were described as mean and standard deviation or median and interquartile range. The associations between maternal and neonatal vitamin D metabolites (total, free, bioavailable 25OHD, vitamin D binding protein) were examined via Pearson correlation or Spearman correlation coefficient. Separate Generalized linear models (GLMz) were used to examine the associations between maternal and cord vitamin D metabolites with the last ultrasound biometry and anthropometric measures at birth. Then, the associations were controlled for potential covariates based on previous literature (pre-pregnancy BMI, gestational age at delivery or gestational age of last ultrasound scan and fetal sex) (15, 22, 28). GLMz was chosen because of its robustness as estimator in the absence of normality. Identity link function and gamma with log link were used. Deviance value, value/df and Akaike's Information Criterion (AIC) were used in model selection. Statistical significance was set at $p<0.05$.

RESULTS

A total of 77 healthy mothers-neonates pairs were recruited. Majority (74.0%) of the respondents were highly educated Chinese, 68.4% and 21.1% of them were employed and self-employed, respectively (Table I). Despite all pregnant women consuming vitamin D-containing supplements, more than half (61.0%) of them were found to be vitamin D insufficient (<50 nmol/L). Mean maternal total 25OHD was significantly lower than cord total 25OHD (mean 47.1 ± 14.7 nmol/L vs 57.0 ± 17.1 nmol/L, $p<0.0001$). Thus, 35.1% of neonates were found to be vitamin D insufficient.

In both maternal and cord samples, total 25OHD was positively correlated with VDBP ($r=0.239$ and $r=0.276$ respectively) but not correlated with free and bioavailable 25OHD. Maternal total 25OHD was strongly correlated with cord total 25OHD ($r=0.731$, $p<0.0001$) and modestly correlated with cord VDBP ($r=0.371$, $p<0.01$). Maternal VDBP was positively correlated with cord VDBP but negatively correlated with cord free 25OHD and cord bioavailable 25OHD ($r=0.455$, -0.366 and -0.393, respectively). Maternal free 25OHD and maternal bioavailable 25OHD were both modestly correlated with cord free 25OHD and cord bioavailable 25OHD (Table II).

Maternal total 25OHD was negatively associated with birth weight after adjusting for covariate (Table III). However, mothers with a lower total 25OHD was associated with larger head circumference in newborn ($B=-0.042$, $P=0.011$) after the adjustment. Similarly, maternal 25OHD was found negatively associated with biparietal diameter (BPD) in the crude model ($B=-0.001$, $P=0.006$). However, this association did not reach significance ($B=-0.001$, $P=0.065$) after adjusting for cord total 25OHD, pre-pregnancy BMI, fetal sex and gestational age. Maternal total, free 25OHD and bioavailable 25OHD were not associated with length and Ponderal index in both crude and adjusted models. Cord total 25OHD was associated with BPD in the crude model. However, the association attenuated after adjusting with covariates and maternal total 25OHD (Table IV). There were no associations between cord total, bioavailable and free 25OHD concentration with any anthropometric measurements at birth.

DISCUSSION

We described a suboptimal vitamin D status among

Table I: Maternal and neonatal characteristics (n=77)

	mean ± SD or median(Q1-Q3) or n (%)
Maternal Characteristic	
Ethnicity	
Chinese	57 (74.0)
Indian	18 (23.4)
Malay	2 (2.6)
Household Income: High (>RM5000)	44 (63.8)
Education level: Degree or higher,	70 (90.9)
Age (years)	33.4 ± 3.7
Pre-pregnancy weight (kg)	54.9 ± 8.1
Height (m)	160.5 ± 6.5
Pre-pregnancy body mass Index (kg/m^2)	21.1 ± 2.6
Nulliparous	28 (38.4)
Vitamin D supplement intake	
<10ug/day	33(42.9)
≥ 10ug/day	44(57.1)
Total 25OHD (nmol/L)	47.1 ± 14.7
Vitamin D status: Insufficiency (<50nmol/L)	47 (61.0)
Vitamin D binding protein (umol/L)	8.4 (3.7-18.4)
Bioavailable 25OHD (nmol/L)	0.8 (0.4-1.8)
Free 25OHD (pg/ml)	2.7 (1.5-5.7)
Neonates characteristics	
Infant sex:	
Female	38(49.4)
Male	39 (50.6)
Last ultrasound Biparietal diameter (mm)	93.5(91.5-95.2)
Gestational age at last ultrasound (week)	38.0± 1.1
Gestational age at birth (week)	38.8 ± 0.9
Birth weight (kg)	3.2 ± 0.4
Head circumference at birth (cm)	34.2 ± 1.5
Crown-heel length (cm)	49.5 ± 2.6
Ponderal Index	25.6 (24.2-27.5)
Total 25OHD (nmol/L)	57.0 ± 17.1
Vitamin D status (<50nmol/L)	27 (35.1)
Vitamin D binding protein (umol/L)	3.6 (2.3-7.0)
Bioavailable 25OHD (nmol/L)	2.5 (1.3-3.5)
Free 25OHD (pg/ml)	7.3 (4.2-11.5)

Table III: Associations of maternal total 25OHD, free 25OHD and bioavailable 25(OHD) with infant anthropometric measurements

	Birth weight (g)[a]		HC (cm)[a]		BPD (mm)[b]	
	B (SE)	p	B (SE)	p	B (SE)	p
Maternal Total 25OHD						
Crude	-5.5 (3.3)	0.091	-0.02 (0.01)	0.074	-0.001 (0.0004)	0.006
Adjusted[c]	-10.1 (4.4)	0.023	-0.04 (0.02)	0.011	-0.001 (0.0006)	0.065
Maternal Free 25OHD						
Crude	2.7 (9.9)	0.786	0.003 (0.04)	0.923	0.001 (0.0013)	0.561
Adjusted[d]	5.9 (9.9)	0.554	0.007 (0.04)	0.855	0.001 (0.001)	0.553
Maternal Bio 25OHD						
Crude	7.6 (30.3)	0.804	0.004 (0.11)	0.974	0.002 (0.004)	0.580
Adjusted[e]	15.6 (30.3)	0.608	0.004 (0.11)	0.894	0.002 (0.004)	0.648

HC, Head circumference; BPD, Biparietal diameter; Bio, Bioavailable
[a]Values from generalized linear model with normal distribution and identity link function
[b]Values from generalized linear model with gamma distribution and log link function.
[c]Adjusted for cord total 25OHD, maternal pre-pregnancy Body Mass Index, infant sex and gestational age.
[d]Adjusted for cord free 25OHD, maternal pre-pregnancy Body Mass Index, infant sex and gestational age.
[e]Adjusted for cord bioavailable 25OHD, maternal pre-pregnancy Body Mass Index, infant sex and gestational age.

pregnant women of high social economic status in Kuala Lumpur, Malaysia. Our estimate of 61% of maternal plasma with total 25OHD <50 nmol/L was higher than that reported among Chinese pregnant women in Singapore, where at the similar latitude as our study (Kuala Lumpur: 3.1390 N°; Singapore: 1.3521N°) (29). The high prevalence of maternal vitamin D insufficiency reported in this study was comparable with studies conducted among pregnant women at Poland (30), India (17) and United Kingdom (31). In this study, we did not intend to assess the determinants of vitamin D status. However, high vitamin D insufficiency reported in our study suggests that factors other than latitude, for example hours spent under the sun and dietary vitamin D intake, can be important determinants of vitamin D status in our study populations.

The strong association between cord and maternal total 25OHD at delivery reported in our study was consistent with findings from the previous studies (30, 32). Most of the studies reported lower total 25OHD level in cord

Table II: Correlations with maternal and cord Total 25OHD, VDBP, Free 25OHD and Bioavailable

	M Total 25OHD	M VDBP	M Free 25OHD	M Bio 25OHD	C Total 25OHD	C VDBP	C Free 25OHD	C Bio 25OHD
M Total 25OHD	1	0.239[c]	0.067	0.115	0.731[a]	0.371[b]	-0.013	-0.29
M VDBP		1	-0.889[a]	-0.840[a]	0.228	0.455[a]	-0.366[b]	-0.393[b]
M Free 25OHD			1	0.971[a]	-0.031	-0.427[a]	0.422[a]	0.460[a]
M Bio 25OHD				1	-0.023	-0.401[b]	0.435[a]	0.450[a]
C Total 25OHD					1	0.276[c]	0.179	0.159
C VDBP						1	-0.869[a]	-0.872[a]
C Free 25OHD							1	0.992[a]
C Bio 25OHD								1

M, maternal; C, cord; Bio, Bioavailable; VDBP, Vitamin D binding protein
[a]p<0.0001 [b]p<0.01 [c]p<0.05

Table IV: Associations of cord total 25OHD, free 25OHD and bio-available 25(OHD) with infant anthropometric measurements

	Birth weight (g)[a]		HC (cm)[a]		BPD (mm)[b]	
	B (SE)	p	B (SE)	p	B (SE)	p
Cord Total 25OHD						
Crude	-1.3 (2.9)	0.652	-0.04 (0.01)	0.683	**-0.001 (0.0004)**	**0.023**
Adjusted[c]	7.8 (4.2)	0.063	0.03 (0.02)	0.092	0.0001 (0.0006)	0.497
Cord Free 25OHD						
Crude	-9.6 (8.1)	0.242	-0.01 (0.03)	0.619	0.0001 (0.001)	0.813
Adjusted[d]	-7.3 (8.4)	0.389	-0.01 (0.03)	0.727	0.0001 (0.001)	0.646
Cord Bio 25OHD						
Crude	-25.9 (25.1)	0.303	-0.04 (0.09)	0.652	0.0001 (0.003)	0.970
Adjusted[e]	-19.7 (25.5)	0.440	-0.03 (0.09)	0.754	0.002 (0.003)	0.497

HC, Head circumference; BPD, Biparietal diameter; Bio, Bioavailable
[a]Values from generalized linear model with normal distribution and identity link function
[b]Values from generalized linear model with gamma distribution and log link function.
[c]Adjusted for maternal total 25OHD, maternal pre-pregnancy Body Mass Index, infant sex and gestational age.
[d]Adjusted for maternal free 25OHD, maternal pre-pregnancy Body Mass Index, infant sex and gestational age.
[e]Adjusted for maternal bioavailable 25OHD, maternal pre-pregnancy Body Mass Index, infant sex and gestational age.

samples than in maternal samples which cord level of total 25OHD was about 75% of maternal level at term. It is noteworthy to mention that, these studies used liquid chromatography (LC) method to assess 25OHD level (16, 33, 34).In contrast, we found significantly higher neonatal total 25OHD than maternal total 25OHD. It is likely that this discrepancy is due to the use of chemiluminescent immunoassay (CLIA) LIAISON in measuring total 25OHD, as higher neonatal total 25OHD than maternal total 25OHD was also reported in several previous studies where CLIA LIAISON method was used (23, 30, 35). CLIA LIAISON is a non-extracting method which may not fully dissociate 25OHD from VDBP especially in maternal samples that have high VDBP concentrations. Nonetheless, CLIA LIAISON method has a high throughput, satisfactory precision and accuracy, as compare to LC method. In addition, the discrepancy may be related to the maternal vitamin D status. Novakovic et al. (36) in their analysis showed significantly higher neonatal 25OHD than maternal 25OHD was observed at lowest quartile of maternal 25OHD (25OHD ≤46nmol/L) but was not apparent at higher maternal 25OHD. In this study, approximately 61% of pregnant women had 25OHD ≤50nmol/L. Thus, higher neonatal 25OHD than maternal 25OHD is expected

An early study by Bouillon et al. (37) showed a strong correlation between maternal and cord free 25OHD (r=0.86) suggesting facilitated transfer of free 25OHD through the placenta. Nevertheless, our results showed only modest correlations (r≈0.4) between maternal bioavailable and free 25OHD with cord bioavailable and free 25OHD. This finding did not suggest transfer of maternal bioavailable and free 25OHD to the fetus through the placenta.

In our studies, we observed the confounding effects of cord total 25OHD on the associations of maternal total 25OHD with several neonatal anthropometric measurements. For instances, maternal total 25OHD was not associated with all the neonatal anthropometric measurements except biparietal diameter in the crude mode. However, the associations between maternal total 25OHD with birth weight and head circumferences were significant after adjusting for cord total 25OHD and other variables. The finding that maternal total 25OHD but not cord total 25OHD was associated with birth weight is in good agreement with Chun et al. (33). However, this finding was inconsistent with the finding by Lykkedegn et al. (16), which they found cord total 25OHD but not maternal total 25OHD was associated with birth weight. However, two studies showed both maternal and cord total 25OHD were not associated with birth weight (30, 38). The biological basis to explain the associations between vitamin D and neonatal anthropometric measurements at birth remains unclear. The significant association between maternal but not cord total 25OHD with birth weight suggests that the association is likely to be mediated by the placenta. Maternal 25OHD may affect fetal growth by regulating placental expression of specific genes that are involved in fetal growth. In agreement with this, a previous study has shown that vitamin D plays an important role in regulating placental amino acid transporters (39).

The negative association between maternal 25OHD and birth weight may be attributed by the interaction effect of 25OHD with variables such as pre-pregnancy BMI and infant's sex. Sauder et al. (15) reported a significant interaction between 25OHD with pre-pregnancy BMI for total mass. They found that among women with lower pre-pregnancy BMI, an increase in 25OHD was associated with a decrease in birth weight, whilst among women with higher pre-pregnancy BMI, an increase in 25OHD was associated with an increase in birth weight. Our study population has low pre-pregnancy BMI (mean ± SD: 21.1 ± 2.6 kg/m^2). It is therefore not surprising that we observed negative associations between maternal total 25OHD with birth weight. In a study by Eggemoen et al. (28), there was sex differences in the associations of maternal total 25OHD with birth outcomes. Their study showed maternal total 25OHD was negatively associated with the sum of skinfolds in males but not in females. Nonetheless, our study had limited power to analyse the potential interactions between 25OHD with these variables.

Several studies have suggested that free 25OHD was better correlated with the markers of vitamin D and the endocrine system as well as bone health than total 25OHD in both the pregnant (40) or non-pregnant state (5). However, the evidence is still inconsistent (5). Recently, a longitudinal study by Gustafsson et al. (11)

reported that both total and free 25OHD in the second trimester was negatively associated with birth weight in the crude model, but the associations attenuated after it was adjusted for several variables. Our finding is consistent with theirs in which we found no associations between maternal and cord free and bioavailable 25OHD with birth weight and other parameters in crude and adjusted models.

This was a preliminary study conducted in a private hospital setting. The sample size was small. Thus, we had a limited power to observe significant associations. Besides, the results might not generalise to larger Malaysian population. However, this preliminary study provides new insights into the associations between free and bioavailable 25OHD with neonatal anthropometric measurements. Free and bioavailable 25OHD were estimated based on published mathematical models that utilised average binding coefficient of VDBP instead of direct measurement. However, preliminary data from commercially available immunoassay showed good correlations between direct measured free 25OHD with calculated free 25OHD (40), (41). Different VDBP genotypes may have distinct VDBP binding affinity for 25OHD. Monoclonal VDBP assay used in this study was sensitive to VDBP variant GC1f. This may result in lower measured VDBP level in monoclonal VDBP assay compared to the polyclonal VDBP assay, thus bias in measuring VDBP level in genetically mixed populations (41). However, our study population was homogenous. The use of monoclonal assay should not have any great impact on our results. Likewise, measuring VDBP levels by polyclonal assay may differ according to the assay technique and vary from batch to batch. There is a need for standardization of the assay measuring free 25OHD and VDBP.

Despite these limitations, we comprehensively assessed total, free and bioavailable 25OHD in maternal and cord blood, attempting to understand their correlations and provide insight into maternal-fetal transfer of vitamin D metabolites. To the best of our knowledge, this is the first study to examine the associations of both maternal and cord free and bioavailable 25OHD with neonatal anthropometric measurements. We assessed the associations of maternal and cord vitamin D metabolites with outcomes concurrently. We then adjusted these associations with corresponding maternal or cord vitamin D metabolites to examine whether maternal or cord vitamin D metabolites, which are associated with birth outcomes. Although the observed associations between vitamin D metabolites and birth outcomes are small, these associations may reflect changes in fetal programming which may have long-term health consequences.

CONCLUSION

Our study reported a suboptimal vitamin D status in pregnant women and neonates from high social economic status. Also, we demonstrated that maternal total 25OHD but not cord total 25OHD are associated with birth weight and head circumferences. These findings suggested that the effect of 25OHD on birth weight and head circumferences are likely to be mediated through the placenta. Our findings did not suggest causative relationships between total 25OHD and birth outcomes. However, the suggested associations could encourage further research in larger sample size. Notably, future research might lead to the establishment of the causative relationships and plausible mechanisms between maternal and cord 25OHD with neonatal anthropometric measurements at birth.

ACKNOWLEDGMENTS

We are grateful to all the women who participated in this study. We acknowledge the staff at Fetal Medicine and Gynaecology Centre (FMGC) for their assistance in data collection and the midwives at Pantai Hospital Kuala Lumpur for assisting in collecting umbilical cord blood samples.

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