Maternal and umbilical cord 25(OH)D levels at delivery and identification of factors affecting the correlation: a prospective observational study from a northern Emirate.

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Abstract

25-hydroxyvitamin-D (25(OH)D) level in the fetus entirely depends on the transport over placenta which is assumed to be obstructed with maternal levels <25nmol/L. Design Observational study. Setting Prospectively enrolling from September to December 2021 in a tertiary governmental hospital in a northern Emirate. Population Admission for spontaneous and elective labor. Methods 25(OH)D was analyzed in maternal serum at admission, respectively, in cord blood after delivery. Main outcome measures Factors affecting maternal and fetal 25(OH)D levels and the correlation between the two at delivery. Results 303 women were enrolled and 237 had complete maternal/umbilical cord blood samples. 138 (47.7%) of women were diagnosed with deficiency (25(OH)D<50nmol/L), whereas only 34 fetuses (13.8%) were deficient (25(OH)D<30nmol/L). The mean difference between maternal and cord blood 25(OH)D was negative in 91% of cases (-16.27nmol/L, SD=13.36). The correlation between maternal/umbilical cord levels was excellent (r = 0,906, p<0,000). After dividing into subgroups of maternal 25(OH)D levels, BMI, diabetes yes/no and delivery-mode the correlation was consistent, although the correlation coefficients in the subgroups of maternal 25(OH)D levels were lowered for all groups. Of factors studied, only the supplementation dose affected the maternal 25(OH)D level. 25(OH)D <50nmol/L was not associated to an increased risk for diabetes, preterm labor, preterm-rupture-of-membranes or low Apgar-score. Conclusion We found a higher 25(OH)D level in cord blood with consistent correlation to maternal levels. Of the studied factors only supplementation dose had significant impact on the maternal level. 25(OH)D <50nmol/l were not associated to increased risk for preterm-labor, preterm-rupture-of-membranes, diabetes or low AS.

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Running title: 25(OH)D in maternal and umbilical cord blood.

ABSTRACT

Objective 25-hydroxyvitamin-D (25(OH)D) level in the fetus entirely depends on the transport over placenta which is assumed to be obstructed with maternal levels <25nmol/L.

Design Observational study.

Setting Prospectively enrolling from September to December 2021 in a tertiary governmental hospital in a northern Emirate.

Population Admission for spontaneous and elective labor.

Methods 25(OH)D was analyzed in maternal serum at admission, respectively, in cord blood after delivery.

Main outcome measures Factors affecting maternal and fetal 25(OH)D levels and the correlation between the two at delivery.

Results 303 women were enrolled and 237 had complete maternal/umbilical cord blood samples. 138 (47.7%) of women were diagnosed with deficiency (25(OH)D<50nmol/L), whereas only 34 fetuses (13.8%) were deficient (25(OH)D<30nmol/L). The mean difference between maternal and cord blood 25(OH)D was negative in 91% of cases (-16.27nmol/L, SD=13.36). The correlation between maternal/umbilical cord levels was excellent (r = 0,906,p <0,000). After dividing into subgroups of maternal 25(OH)D levels, BMI, diabetes yes/no and delivery-mode the correlation was consistent, although the correlation coefficients in the subgroups of maternal 25(OH)D levels were lowered for all groups. Of factors studied, only the supplementation dose affected the maternal 25(OH)D level. 25(OH)D <50nmol/L was not associated to an increased risk for diabetes, preterm labor, preterm-rupture-of-membranes or low Apgar-score.

Conclusion We found a higher 25(OH)D level in cord blood with consistent correlation to maternal levels. Of the studied factors only supplementation dose had significant impact on the maternal level. 25(OH)D <50nmol/l were not associated to increased risk for preterm-labor, preterm-rupture-of-membranes, diabetes or low AS.

Keywords: Vitamin D, 25(OH)D, pregnancy, fetal cord blood, diabetes, obesity.

Tweetable abstract: 25(OH)D level in cord blood is higher than in maternal blood with a strong correlation not influenced by maternal levels, delivery mode, BMI or diabetes.

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INTRODUCTION

25-hydroxy vitamin D (25(OH)D) is the most abundant form of vitamin D and is recommended for measurement of the total body concentration of the vitamin (1). The definition of hypovitaminosis D in pregnancy is having a 25(OH)D below 50nmol/l, whereas in newborns the cutoff for deficiency is 30nmol/L. However, it has been argued that the ideal level in adults should be >75 nmol/L (30 ng/mL) (2). Vitamin D is essential to the human body. Historically, the vitamin is known for its role in calcium and phosphate metabolism, with deficiencies leading to rickets and osteomalaci. As important, upcoming data have shown that vitamin D is linked to numerous essential functions such as reducing inflammation, modulation of the neuromuscular system, regulating glucose metabolism and cell growth (by modulating the genes regulating cell proliferation,

differentiation and apoptosis) (3,4) In pregnancy low vitamin D has been associated with increased risk for preeclampsia, preterm rupture of membranes, small for gestational age fetus, and dystocia but according to WHO the evidence is low and more trials are warranted (2,5,6). Importantly, the fetal level of vitamin D entirely depends on 25(OH)D transfer over the placenta. It is a general assumption that the fetal vitamin D level is lower than the maternal and that there is no transport of 25(OH)D over the placenta with low maternal levels, though of low grade evidence (5,7,8). In the United Arab Emirates, up to 69% of pregnant women has 25(OH)D < 30nmol/L, and the awareness of the importance, also in well-educated women is low (9).

This study aimed to study the maternal and umbilical cord levels of 25(OH)D at delivery and to investigate if obesity, diabetes, amount of supplementation, delivery mode influenced the maternal level and the correlation between maternal and umbilical cord blood levels. Secondary to study certain risks associated with 25(OH)D levels < 50nmol/L.

MATERIALS AND METHODS

This prospective observational study was conducted from 27th September until 31st December 2021 at Sheikh Khalifa Hospital, Women and Children, Ajman, UAE, in collaboration with Lund University, Lund, Sweden.

Procedure

Inclusion criteria were: admission for delivery and signed informed consent from the mother, and if married also the father, to analyze 25(OH)D and to enter the medical records from both the mother and the newborn. Women were excluded if they did not speak Arabic, English or Urdu. Maternal venous blood was collected together with the routine blood sampling at admission to the labor ward and for the fetus, paired umbilical cord blood samples were collected directly after delivery in pre-heparinized syringes on unclamped cord. Before discharge, the mothers answered a questionnaire in the presence of research officials regarding education, physical activity, time spent outside, compliance, importance of vitamin D supplementation to the baby and her plan to breastfeed (Appendix 2, not all data processed for this article). Data was entered into a specific study database from the electronic medical record (Cerner, Missouri, USA).

Biochemical analysis

Despite the challenging and ongoing discussions on how to measure the level of vitamin D current guidelines recommend measurement of 25(OH)D (1). The maternal and umbilical cord blood were sent directly to the hospital's central laboratory for analysis by an electrochemiluminescence binding assay using Liaison 25-hydroxy-vitamin D TOTAL. The laboratory is internationally certified by Analytics Professional (CAP) and has accreditation from The International Organization for Standardization (ISO). The cord blood gases and electrolytes were analyzed directly on the labor ward by*a* stationary blood gas analyzer (ABL90, Radiometer, Copenhagen, Denmark).

Statistical analysis

One-way ANOVA was used for group comparison of continuous variables and Fisher's exact test for frequencies. Pearson's correlation coefficients (r) were calculated. Multiple Linear Regression was used for identifying relation between the independent and dependent variables. Binary logistic regression was used to measure the association between continuous vitamin D levels and the probability of having diabetes, preterm delivery, preterm rupture of membranes and 5-minute Apgar score (AS) <7 and is presented with odds ratios (OR and the 95% confidence interval). Descriptive statistics were presented as mean with standard derivation (SD) and medians with interquartile range (IQR). All tests were two-tailed and a P-value <0.05 was considered statistically significant. Analyses by IBM SPSS 28 (SPSS Inc. Chicago, Illinois, USA).

Ethics

The study was approved before the start by the Ministry of Health and Prevention, United Arab Emirates, with diary number: MOHAP/DXB-REC/SSS/No. 79/2021.

RESULTS

Of the 303 women who were enrolled, 237 had complete mother/umbilical cord blood data. 138 (47.7%) women had vitamin D deficiency (< 50nmol/L) at delivery whereas only 34 (13.8%) of the cord-blood samples showed levels under the recommended cut-off for newborns (< 30nmol/L) (10). The mean difference between maternal venous blood and cord blood 25(OH)D was negative (higher levels in the cord blood compared to the maternal blood) in 91% of cases (-16.27nmol/L, SD=13.36), Fig 1. The difference was not significantly associated with maternal 25(OH)D level, placenta weight or delivery mode ($p > 0.493$). After dividing the women into three groups, <25nmol/L (n=64), 25-49.9nmol/l (n=74) and [?]50nmol/L (n=151) we did not find significant difference in maternal age, gestational age at delivery, fetal weight, level of ionized calcium ($Ca2^+$) in the cord blood, maternal body-mass-index (BMI), nationality, exposure to sunlight, frequency of diabetes or delivery mode, Table 1. Multiple regression with maternal 25(OH)D as the dependent variable and supplementation dose, age, BMI, nationality, education, exposure to sunlight, diabetes as independent variables showed that only the dose of supplementation (p <0.001) had a significant influence on maternal 25(OH)D, Table not shown.

The correlation between maternal and umbilical cord 25(OH)D was for the whole cohort 0.906 ($p < 0.001$). Subdivision into different levels of maternal 25(OH)D, delivery mode, diabetes and BMI showed a lower correlation coefficient for all three subgroups of maternal levels. For all other calculations the correlation was excellent (Table 2). There was no correlation between placenta weight or hematocrit to the umbilical cord level of 25(OH)D (r = 0.193, $p = 0.180$, respectively r = -0.063, $p = 0.353$).

The correlation between maternal and umbilical cord 25(OH)D was not to a clinical point of view affected by the supplementation dose (none supplementation n = 18, r = 0.948; 10.000UI/week n = 73, r = 0.853; 20.000UI/week n = 38, r = 0.805; all P values < 0.000). For those without information about supplementation (n = 106) the correlation coefficient was 0.938 (P < 0.000). The correlation between umbilical cord $Ca2^+$ and supplementation dose was non-significant (n = 141, r = 0.000, $p = 0.997$).

The risk for preterm labor (OR 1.725, 95%CI: 0.808-3.683) preterm rupture of membranes (OR 1.538, 95%CI: 0.678-3.491), low AS (3.465, 95%CI 0.310-38.777) with 25(OH)D levels < 50nmol/L was not increased whereas the risk for diabetes was significantly decreased (OR 0.470, 95 CI 0.252-0.879).

DISCUSSION

Main findings

This study shows that 25(OH)D levels in umbilical cord blood are higher than levels in maternal blood. The correlation between the maternal and umbilical cord blood is solid and consistent over the range of maternal levels. Only the supplementation dose significantly impacted the maternal 25(OH)D level. Low maternal 25(OH)D levels were not associated with a risk for premature labor, premature rupture of the membranes, diabetes, or low AS.

Interpretation

We analyzed total 25(OH)D ($25(OH)D_2$ + $25(OH)D_3$). There are different metabolites of vitamin D, all with various stability and biological activity, mainly explained by the affinity to the vitamin D receptor (1). Although new arrays for measuring the different vitamin D metabolites, the international recommendation is still to measure the cumulative level by quantifying the amount of 25(OH)D (1). Total 25(OH)D has a long half-life-time giving only minor variations within short periods, and it shows a significant response to supplementation intake (increased $25(OH)D_{2,}$) but mainly to dermal vitamin production (increase $25(OH)D_3$) (11). Interestingly, genetic factors are shown to play a role in the uptake of $25(OH)D_2$ why it is speculated if some require a higher supplementation dose than others, but when it comes to transferring to the fetus, Novakovic et al., concludes that maternal circulation 25(OH)D overrides genetic factors (12). Due to the relatively low percentage of patients with documented supplementation, we could not draw any conclusions except that supplementation in general increases the maternal 25(OH)D level. In contrast BMI, nationally, education,

daily exposure to sunlight, or diabetes did not significantly influence. How to optimize supplementation is a subject for future research within personalized medicine and big data analytics.

Some of our participants received a high dose of weekly vitamin D. The Vitamin D supplementation could be as high as 20.000 IU/week plus 800 IU daily from the prenatal vitamin tablets, making the accumulated dose 25.600 IU/week or 3657 IU/day. The highest dose of supplementation evaluated during pregnancy is 4000 IU/day; a higher amount is thought to cause fetal hypercalcemia (13). We had two patients with individualized vitamin D supplementation; one had 8071 IU/day and the other 5085 IU/day, but none of the newborns had hypercalcemia (results not shown).

From the questionnaire, we know that 98% of the enrolled women were well aware of the importance of supplementation, assuming most were taking vitamin D without specifying how much. Recently, Izzeldin et al. described a high incidence of 25OH)D levels <25nmol/L in pregnant women in the United Arab Emirates (69%), whereas in our study is was 28% (9). The time spent outside, the analysis method or genetic factors cannot explain the difference (approximately 2% of women spent more than 30 minutes outside per day, the same laboratory used, the same population). In their study, 12% understood the importance of vitamin D and enrolling in the second trimester theoretically before the first antenatal care visit and discussion of supplementation; many of the ladies could be deficient at enrollment. All the consultants and specialist (n=17) in the Obstetrical/Gynecology department at our hospital answered the questionnaire before study start. Within the collegium there was no consensus on how to screen, treat or follow-up the level of 25(OH)D (data not shown). This highlights the need to implement a straightforward guideline where early screening for vitamin D deficiency, women's education, and how to give supplements are accurate.

To avoid newborn vitamin D deficiency (defined as <30nmol/L) it is suggested that maternal levels have to be >50 nmol/l (8). Results from our study contradict this statement. With maternal levels <50nmol/L no newborn was born with levels under 40.5nmol/L. Only if the mother had levels <23nmol/L was a risk for deficiency in the newborn.

From numerous randomized trials, it is known that cord blood 25(OH)D increases after supplementation during pregnancy, although an increase does not consistently follow an equal rise in the mother (14). We speculated if our result was due to the timing of maternal 25(OH)D measurement. We could not explain the higher cord blood level by the placenta weight, cord blood hematocrit, or mode of delivery (15). Out of 40 studies, Saraf et al. found only three studies describing a negative ratio between maternal and fetal blood (10). One was analyzing 1,25-hydroxyvitamin D making a comparison to our study futile. The other two studies were small but with designs very similar to ours i.e., maternal/fetal measurements close to each other (12,16). There is evidence for fluctuation of the different vitamin D metabolites during pregnancy. Fluctuation and months between maternal and newborn 25(OH)D measurement, typical for most studies, interprets a correlation as troublesome.

Compared to other studies, we successfully obtained a high number of paired maternal and umbilical cord blood 25(OH)D samples within a specific and narrow time frame. The correlation between maternal and fetal levels was strongly correlated and independent of delivery mode and maternal characteristics. We were not able to show any clinical difference in the correlation over the range of different maternal levels as shown by Wegienka et al. and Rabbani et al. Comparison between methods showed that Wegienka et al. recruited patients throughout the whole third trimester and also included 25(OH)D levels from the second trimester. In Rabbian's study, only 19% had received supplements before blood sampling (7,17).

The evidence for an association between low 25(OH)D in the mother and complications during pregnancy such as obesity, diabetes, preterm rupture of the membranes, and dystocia are for discussion. Published results are based on inhomogeneous observation studies, commonly without specifying the timing for sampling (7–9). Although this study was not an RCT we did not add evidence for an association between vitamin D deficiency and complications in pregnancy or delivery.

Strenths : The study was conducted in a high endemic region and with a high number of paired maternal and umbilical blood samples taking within a specific and narrow time frame. Analysis of 25(OH)D was

done directly after sampling. Data was not retrieved from a register but directly from the EMR, avoiding methodological errors.

Weakness : Nearly 50% of the women were un-booked, i.e., they had no previous antenatal care visits in our hospital, implying that specific antenatal data was missing. Due to hemolysis of the blood, it was impossible to get the results of 25(OH)D in 66 cases.

Conclusion

Contradicting previously published results, we found that the level of 25(OH)D was highest in cord blood. A low maternal level did not obstruct the transport over the placenta. Also, the odds for premature delivery, premature rupture of membranes, low AS, or diabetes were not increased with 25(OH)D levels <50nmol/L. Considering that 47% of mothers were deficient, the study highlights the need for a standardized guideline for screening, treating, and following up pregnant women, especially in high-risk areas.

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DISCLOSURE OF INTEREST

None

AUTHOR CONTRIBUTIONS

MJ, MB, SB and NW designed the study. MJ, MB, SB collected the data. MJ, PEI and NW processed the data. All authors interpretated the results. MJ, NW drafted the manuscript, critical revision by PEI, MB, SB. All authors agreed on approval of the manuscript before submission.

Ethics

The study approved by the Ministry of Health and Prevention, United Arab Emirates, diary number: MOHAP/DXB-REC/SSS/No. 79/2021.

FUNDING

Sheikh Khalifa Hospital, Women and Children, Ajman, founded the costs of the 25(OH)D analysis.

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